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On the metabolic origin of cancer: substances that target tumor metabolism.

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Abstract.

Work from our group and others clearly suggest the key role of altered metabolism in cancer. The goal of this review is to summarize current knowledge on cancer metabolism, draw hypothesis explaining metabolic alterations and associated gene changes. Most importantly, we indicate a list of possible pharmacological targets. In short, tumor metabolism displays mixed glycolysis and neogluogenesis features; most glycolitic enzymes are activate, but the pyruvate kinase and the pyruvate deshydrogenase are inhibited. This would result from an activation of their specific kinases, or from the inactivation of phosphatases, such as PP2A, regulated by methylation. In parallel, the phosphatase failure would enhance "tyrosine kinase receptor" signals, as occurs with oncogenes. Such signaling pathways are similar to those activated by insuline, or IGF- Growth hormone; they control mitosis, cell survival, carbohydrate metabolism. If for some reason, their regulation fails (oncogenes, PP2A methylation deficit, enhanced kinases…) a typical tumor metabolism starts the carcinogenic process. We also describe changes in the citric acid- urea cycles, polyamines, and show how body stores feed tumor metabolic pathways above and below "bottlenecks" resulting from wrongly switched enzymes. Studying the available literature, we list a number of medications that target enzymes that are essential for tumor cells. Hoping to prevent, reverse or eradicate the process. Experimental data published elsewhere by our group, seem to confirm some of these assumptions.

Keywords: Cancer. Oncogenes. Tyrosine kinase receptor. PP2A methylation. Pyruvate kinase M2

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Introduction

Normally, lipid and muscle protein stores decrease after fasting for example, when one needs to synthesize nutriments: ketone bodies and glucose by neoglucogenesis. However, tumors utilize such stores for supporting an elevated very special glycolysis, with lactate production and release, a process discovered by Warburg [1]. It is like if neoglucogenesis and glycolysis switches were jammed in tumors. Glycolysis is on, but pyruvate kinase (PK) and pyruvate dehydrogenase (PDH) are both at rest, like for a neoglucogenic metabolism, see ref [2].

Hence, all the initial part of glycolysis operates, with its glyceraldehyde dehydrogenase step that needs NAD+, it will come from the conversion of pyruvate into lactate, by lactate dehydrogenase (LDH), explaining the Warburg effect. Much of the pyruvate needed results from muscle protein proteolysis, usually increased in cancer, via alanine transamination, alanine being an amino acid directly transaminated into pyruvate.

Since PDH is inactive, the junction between glycolysis and the citric acid cycle-oxidative metabolism stops. However, below PK and PDH bottleneck, citrate synthase is particularly active [3-5], NADH probably no longer inhibits it. Citrate synthase will have to get acetyl-CoA from fatty acid β oxidation, while lipid stores mobilize; weight is lost, and this is frequent in patients with cancer.
The other substrate required for citrate condensation, oxaloacetate (OAA), may come from phosphoenol pyruvate (PEP) accumulated above the PK bottleneck, since PEP carboxykinase is a reversible enzyme; other OAA sources involve ATP citrate lyase, MAL dehydrogenase and aspartate transaminase. Pyruvate carboxylase (Pcarb) might have provided OAA, but the enzyme is probably at rest [6], since its activator, acetyl-CoA, decreases because the citrate condensation is particularly active, which leaves even more pyruvate to (LDH). The blockade of PK and PDH by phosphorylation [2], results from the activation of kinases [7, 8] or the inactivation of phosphatases. How did the blockade occur in the particular case of tumors?

We find a possible answer to the question in early observations on the effect of insulin [9]. They show that after pancreatectomie, a total pancreatic extract, protects from steatosis and cancer associated to steatosis, [9-11] because the extract, unlike purified insulin, contains choline derivatives. We know that choline is not only a lipot-

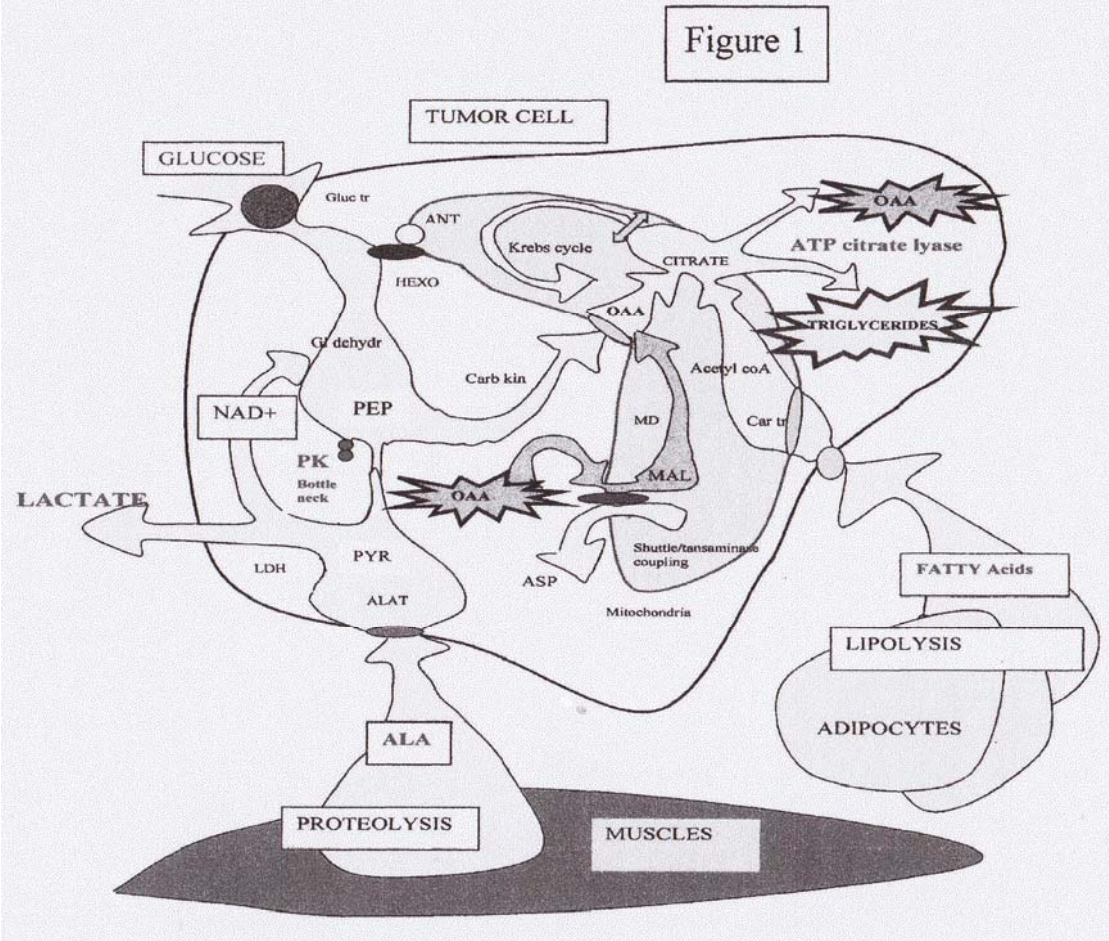

Figure 1 Tumor cell metabolism

The increase of glucose influx in tumor cells, is driven through the glucose transporter (Gluc tr) coupled to hexokinase (Hexo). This enzyme interacts with a mitochondrial complex, and receives the necessary ATP, via the ATP/ADP translocator (ANT). In tumor cells, this increases glycolysis, but the glycolytic flux stops at the pyruvate kinase (PK) "bottleneck" (the M2 dimer remains phosphorylated). Above the neck, the accumulated phosphoenol pyruvate (PEP) feeds in the mitochondria, where it is carboxylated into oxaloacetate (OAA), by PEP carboxy kinase (carb kin); the reaction involves CO2- biotin and GDP. The OAA flux feeds the citrate condensation, reacting with acetyl-CoA. The latter, comes from the β oxidation of fatty acids, since PDH is also at rest in its phosphorylation state, an intense lipolysis takes place. Fatty acids are transported in cells, and then enter the mitochondria via the carnitine/ acetylcarnitine transporter (CAR tr). The citric condensation reaction and the formation of triglycerides via ATP citrate lyase, pulls the flux; triglycerides rather than ketone bodies form. The other ATP citrate lyase product OAA feeds the malate dehydrogense reaction. An analysis of the malate / aspartate shuttle, in relation to transaminases, completes the description see addendum and figure 8C. The decrease of NADH, which activates citrate synthase, may result from a blockade of α ketoglutarate dehydrogenase, closely related PDH. A down stream aconitase inhibition by NO or peroxynitrite would favor the efflux of citrate along the ATP citrate lyase route. Uncoupling proteins decreasing the NADH potential are also probably involved. Below the bottleneck, pyruvate (PYR) will no longer come via pyruvate kinase, which stops converting PEP into PYR; other sources become available, PYR comes essentially from alanine, transaminated by (ALAT). PYR will then give lactate, via lactate dehydrogenase (LDH), in order to form the NAD^+ required by glyceraldehyde-Pdehydrogenase (Gl dehydr), for glycolysis to proceed. Other NAD^+ sources (not shown in the figure) come from glutamate dehydrogenase (GDH), converting α ketoglutarate (α keto) into glutamate (GLU); or Mal dehydrogenase oriented by the OAA flux coming from PEP carboxy kinase and ATP citrate lyase. Muscle proteins are proteolysed, providing much alanine to tumors; lipolysis and proteolysis feed glycolysis: "Tumors burn their host".

ropic factor, but also a methyl source. Moreover, several observations show that a phosphatase, PP2A, is a methyla tion sensitive phosphatase, finding specific targets after methylation of its catalytic unit [12,13]. The poor methylation of PP2A resulting from the choline deficit would then be a possible answer to our question, if the phosphatase failed to dephosphorylate PK and PDH, keeping them in their inactive form, which closes the junction between glycolysis and oxidative metabolism. In parallel, the poorly methylated phosphatase would become unable to counteract insulin-tyrosine kinase signals, which increase, eliciting the influx of glucose, mitosis and cell survival. The brake over the signaling pathway is in this way turned off [14, 15]. The response to insulin or insulin like signals is no longer coherent, the entry of glucose is increased, but there is a bottleneck on the way. After all, most oncogenes act by increasing a given step in the cascade of similar "tyrosine kinase receptor" signals, controlling mitosis and cell survival, leading to a similar non-coherent response and cancer. The perverted tumor metabolism, which operates above and below the PK and PDH bottlenecks, consumes body lipid and muscle protein stores, (schematic representation figure 1). However, one would like to find what links steatosis, the choline deficit, and the occurrence of hepatomas? Steatosis comes from the accumulation of triglycerides (TG), their synthesis and accumulation results from the elevated citrate condensation, via ATP citrate lyase, combined to a poor lipotropic effect of choline, since it is here deficient. In addition to the poor methylation of PP2A, it is possible that steatosis changes the phosphatase localization, leading it to the nucleus for example. Hence, tyrosine kinase signals in the cytosol become particularly active. In the nucleus, where methylations-demethylations processes operate, the phosphatase may find new targets, activating cell cycle proteins as discussed later.

Moreover, the lipogenic citrate condensation would decrease ketone bodies and butyrate, a classical histone deacetylase (HDAC) inhibitor, which induces an epigenetic reprogramming of genes [15, 16]. Methylated promoters are silenced, while adjacent hypomethylated genes are over expressed, stabilizing the tumor cell features at the genetic level.

Whatever is the trigger boosting the tyrosine kinase receptor signals: oncogenes, hormones, metabolism, and mutations, the result is the same: MAP kinases, PI3 kinases, transcription factors, become activate, inducing mitosis, cell survival and anabolism in response to the trigger. The cellular response is carcinogenic if it is not coherent; this occurs if metabolic switches are not in the right position, as discussed for the bottleneck characterizing tumor metabolism. Oncogenes trigger cancer in an equivalent way, enhancing the expression or the action of proteins belonging to the cascade of tyrosine kinase signals that become particularly active.

The observations linking different features of metabolism, epigenetic changes, and cancer, are not a mechanistic causal demonstration, but identify possible carcinogenic routes.

There are many other carcinogenic conditions boosting tyrosine kinase receptor, and the signals they trigger in a non-regulated way. This is the case of Growth hormone (GH), Insulin like growth factor (IGF) effects for example. Normally, GH induces the synthesis and release of IGF from the liver, which stimulates IGF receptors (IGFR) of the "tyrosine kinase receptor" type, eliciting mitosis [17, 18]. An asymmetrical mitosis of a stem cell takes place, since a single daughter cell inherits the mitotic capability, while the other sterile daughter differentiates. Since the IGF- (IGFR) complex is likely to control the mitotic capability, one expects that an IGF binding protein (IGFBP) of strong affinity for IGF will regulate the process. It may perhaps cap the IGF- IGFR receptor complex in one of the daughter cells, which inherits the mitotic capability. An excess of GH- IGF action perturbed by a decrease of IGFBP, would then favor a mitosis, in which both daughter cells inherit receptors and the mitotic capability, this symmetrical mitosis occurs in tumors, a geometric increase of the dividing mass takes place.

In the present review, we shall analyze metabolic features of tumor cells, in order to identify the regulations that fail along the signaling pathways controlling metabolism. The minimal change that characterizes such a metabolic perversion takes place when PK and PDH are at rest like for neoglucogenesis, while citrate synthase remains active. The role of a phosphatase such as PP2A activated by methylation would normally cancel the effect of kinases that block the enzymes unless it is deficient. As for the increase of citrate synthase – ATP citrate lyase activities, they are in relation to down stream inhibitions at isocitrate, or aconitase steps, of the citric acid cycle, and to the decrease of NADH, which normally inhibits citrate synthase. The stabilization of the metabolic perversion at the genetic level and the activation of a mitotic mechanism, in which both daughter cells divide, is an essential feature to consider. These minimal changes are associated to a cascade of other reactions covering the mitochondria shuttle, glutaminolysis, transaminations, the truncated urea cycle, polyamines, the NAD+ source for glycolysis and lactate production etc… The many consequences of this metabolic perversion are beneficial to the tumor and deplete body stores. On the other hand, this terrible scenario results from abnormal interactions between normal metabolic steps. One may then hope to change with adequate drugs, these interactions and reverse the situation. A mixture of drugs to test on animal models led us to a possible

sequential plurithерapy, in which timing and doses have to be determined.

Perverted glycolytic metabolism of tumors

We shall first consider some crucial steps of glycolysis or neoglucogenesis and indicate some alterations found in cancer, see also ref. [2].

The initial observation of Warburg 1956 on tumor glycolysis with lactate production opened this field [1]. Two fundamental findings complete the metabolic picture: the discovery of the M2 pyruvate kinase typical of tumors [19]; and the interaction of tyrosine kinase phosphorylations and signals, with the M2 pyruvate kinase blockade [14, 20, 21].

Glucose transporter, hexokinase

A glucose transporter, driven by the first enzyme of glycolysis, hexokinase, pulls the influx of glucose in cells. In most tissues, this enzyme interacts with the mitochondria ATP/ADP transporter (ANT) and thus, receives efficiently its ATP substrate [22, 23]. As long as hexokinase occupies this mitochondria site, glycolysis is efficient. However, this has another consequence, hexokinase pushes away from the mitochondria site the permeability transition pore (PTP) inhibiting the release of cytochrome C, the apoptotic trigger [24]. The site also contains a voltage dependent anion channel (VDAC) and other proteins, the repulsion of PTP reduces the pore size and cytochrome C cannot be released. Thus, the apoptosome–caspase proteolytic structure does not assemble in the cytoplasm. A typical feature of tumor cells is a glycolysis associated to an inhibition of apoptosis. Tumors overexpress hexokinase 2, which strongly interacts with the mitochondrial ANT-VDAC complex pushing away the PTP pore, which inhibits apoptosis. We know that the liver hexokinase or glucokinase, is different, it does not stick to the mitochondria site and has a lower affinity for glucose. Liver glucokinase and glycolysis work when glucose gets elevated in the blood, whereas brain hexokinase of greater glucose affinity, operates at lower blood glucose concentrations. Because of this difference, brain receives preferentially glucose. The difference between glucokinase and hexokinase amino acid sequences may lead to peptides displacing hexokinase from the mitochondria site; this would render apoptosis possible in tumor cells.

The polyol pathway

A key reaction of glycolysis is the isomerisation of glucose-6-phosphate into fructose-6-phosphate. The isomerisation of glucose into fructose is essential and operated by the enzyme phosphohexose isomerase. However, there is another route for generating fructose from glucose: the polyol pathway, which normally operates when blood glucose gets elevated [25]. The pathway is active in neurons or red blood cells, with membranes permanently equipped with glucose transporters (these cells do not respond to insulin like muscles or adipose tissues by increasing the number of transporters incorporated in the membrane by exocytotic vesicles). The polyol pathway first converts glucose into sorbitol via an aldol reductase, the enzyme requires NADPH. Then sorbitol, converts to fructose via sorbitol dehydrogenase, which generates NADH. A decrease of NADPH may inhibit several enzymes, NO synthase or glutathione reductase. Other are stimulated (NADH oxidase) forming reactive oxygen radicals. Several consequences result from a polyol pathway activaton, in pathological conditions and cancer. Sorbitol accumulates in tissues, it hardly crosses membranes, and pulls in water in cells, swelling takes place. In lens for example, this favors the onset of cataract. Moreover, the increase of reactive oxygen species aggravates the situation. The decrease of reduced glutathion is also a cause of hemolysis. Like glucose, fructose and its metabolites are potent glycation agents, able to react none enzymatically with the amino groups of proteins, forming advanced glycation products. The discovery of a glycated form of hemoglobin in diabetes demonstrated that this non-enzymatic reaction could modify proteins having a slow turnover; this is also the case of collagen. Fighting glycation becomes an essential goal in the therapy of diabetes, heart diseases, renal diseases, retinopathies, neurodegenerative disorders, aging, and cancer.

The fructose2-6 bis phosphate, cAMP regulation of glycolysis

Further, ahead in glycolysis, phosphofructokinase gives fructose 1-6 bisphosphate; glycolysis is stimulated when an allosteric analogue, fructose 2-6 bisphosphate forms. This occurs if cAMP decreases, in response to insulin for example, when glucose increases in the blood. On the contrary, in starvation, glucagon and epinephrine elicit an increase of cAMP, via adenylate cyclase coupled receptors. The cAMP inhibits the formation of fructose 2-6 bisphosphate; consequently, glycolysis decreases, while neoglucogenesis and glycogenolysis increase, cAMP acts as a hunger signal. In tumor cells, the last step of glycolysis (PK) is not active, as if it was switched-off, for neoglucogenesis, in spite of an active glycolysis. We discuss this point below, in relation with the oxidative-citric acid cycle.

The glyceraldehyde P dehydrogenase step

Another important point of control of the glycolytic pathway is glyceraldehyde P dehydrogenase that requires NAD+ in the glycolytic direction. If the oxygen supply is normal, the mitochondria malate/aspartate shuttle operates, forming via malate dehydrogenase the required NAD+ in the cytosol and NADH in the mitochondria; in this process, malate enters the mitochondria and aspartate

gets out. In hypoxic conditions, NAD+ required for glycolysis, comes essentially from the lactate dehydrogenase reaction, converting pyruvate into lactate. This reaction is prominent in tumor cells, even in normoxia; it is the first discovery of Warburg, on cancer metabolism.

The Pyruvate kinase inactivation

The pyruvate kinase (PK) reaction that gives one of the two ATP molecules of glycolysis, converts phosphoenolpyruvate (PEP) into pyruvate, which enters in the mitochondria as acetyl- CoA, starting the citric acid cycle and oxidative metabolism. Fructose 1-6 bisphosphate activates pyruvate kinase, which can only work in the glycolytic direction, from PEP to pyruvate, which implies that neoglucogenesis will use other enzymes for converting pyruvate into PEP. Two enzymes do the job, pyruvate carboxylase (Pcarb) in the mitochondria, and phosphoenol pyruvate carboxykinase (PEP carboxykinase) in the cytosol and mitochondria. They start the gluconeogenic route, by converting pyruvate into oxaloacetate (OAA) and OAA into PEP until glucose, but it is here necessary to inhibit PK, if not, this would bring us back to pyruvate. In this gluconeogenic direction, the PK is phosphorylated and inactive. Well, tumors have a blocked PK that remains phosphorylated (the M2 isoform is a fetal enzyme).

Wrongly switched enzymes in tumor metabolism

In starvation, when cells need nutriments, glycolysis switches to neoglucogenesis while ketogenesis becomes active. Pyruvate dehydrogenase PDH and PK are off, a phosphorylation of the enzymes takes place, presumably via a cAMP-glucagon-adrenergic signal. A similar inactivation occurs in hibernating animals who consume their stores during this period. In parallel, pyruvate carboxylase becomes active. It receives its substrate, pyruvate, essentially from alanine transamination, provided by muscle protein proteolysis. Pyruvate is converted by Pcarb into oxaloacetate, then processed by PEP carboxykinase into PEP etc.., until glucose. In parallel, the mobilization of lipid stores from adipocytes provides fatty acids. cAMP, AMP, and Growth hormone activate a lipase and mediate this effect. Fatty acids increase, give acetyl-CoA after β oxidation, and ketone bodies. The β oxidation of fatty acids takes place in mitochondria, and peroxisomes for very long fatty acids. The process uses protein and lipid stores to form respectively glucose and ketone bodies in response to starvation. Since OAA starts the neoglucogenic route, it is no longer feeding citrate synthase, the citrate condensation stops, while acetyl-CoA in excess forms ketone bodies. Like glucose, ketone bodies, are nutriments for most tissues including brain. Acetyl-CoA and ketone bodies stimulate the Pcarb, and the OAA production, the first step of neoglucogenesis.

In spite of the active glycolysis of tumors, their PDH and PK enzymes are inactive, like for neoglucogenesis. However, one may reactivate them by inhibiting their respective kinases [7,8]. Moreover, tumors display an abnormally elevated citrate synthase activity, which consumes acetyl-CoA [3,4,5]. Hence, the condensation of acetyl-CoA and OAA into citrate pulls the glucose flux in the glycolytic direction; citrate increases, ketone bodies decrease, which blocks Pcarb. In tumors, the OAA needed for citrate synthase will presumably come from PEP; via reversible PEP carboxykinase, and other OAA sources. The quiescent Pcarb will not consume the pyruvate coming via alanine transamination, after proteolysis of protein stores, and even more pyruvate will go to lactatedehydrogenase, giving the lactate released by the tumor, and NAD+ required for glycolysis, at the glyceraldehyde P dehydrogenase step. In tumors, one finds a particular PK, the M2 embryonic enzyme [19, 26, 27] the dimeric, phosphorylated form is inactive, leading to a « bottleneck » between glycolysis and the Krebs cycle. The M2 PK has to be activated by fructose 1-6 bis P its allosteric activator, whereas the M1 adult enzyme is a constitutive active form. Above the bottleneck, the massive entry of glucose, accumulates PEP, we have seen that mitochondria PEP carboxykinase, an enzyme requiring biotine-CO2-GDP converts PEP into OAA. This source of OAA is abnormal, since Pcarb, another biotin-requiring enzyme, should have provided OAA, but the tumor Pcarb, seems to be inactive. Tumors may contain "morule inclusions" of biotin-enzyme [6] suggesting an inhibition of Pcarb, presumably a consequence of the maintained citrate synthase activity, leading to a decrease of acetyl-CoA and ketone bodies that normally stimulate Pcarb. The PEP abnormal source of OAA adds up to OAA coming from aspartate transamination, or via malate dehydrogenase. OAA will then condense with acetyl-CoA, feeding the citric acid–Krebs cycle. Thus, massive amounts of acetyl-CoA will have to feed the condensation reaction; they come essentially from lipolysis and βoxidation of fatty acids, and enter in the mitochondria via the carnitine transporter. This is the major source of acetyl-CoA, since PDH that might have provided acetyl-CoA remains in tumors, like PK, in the inactive phosphorylated form. The blockade of PDH [15] in tumors is reversible, as recently shown (8). The key question is to find out why NADH a natural citrate synthase inhibitor does not switch off the condensation reaction, which drives the influx of metabolites in the tumor cells. The NADH forms via the dehydrogenases of the Krebs cycle and the malate/aspartate shuttle, which build up its concentration. On the other hand, NADH is consumed by the respiratory electron transport chain via mitochondrial complex1 (NADH dehydrogenase).The overall NADH concentration is presumably low in tumor cells, since there are other probable stops in the Krebs cycle. Indeed, α ketoglutarate dehydrogenase is homologous to PDH and may respond to similar controls. When PDH is off, α ketoglutarate dehydrogenase might also be

off (probably via the action of an intermediate kinase). Moreover, the α ketodehydrogenase blockade is associated to a blockade of aconinase by NO, which increases in tumors. Normally, an increase of NADH inhibits the citrate condensation and the lipogenic formation pathway, favoring the ketogenic route associated to neoglucogenesis. Apparently, this regulation does not occur in tumors, since citrate synthase remains active. Probably, in tumor cells, the α ketoglutarate not processed by α ketoglutarate dehydrogenase, forms glutamate, via glutamate dehydrogenase, in this direction the reaction gives NAD+, backing the LDH source. Glutamate also comes from an active glutaminolysis typical of tumors [19]. This favors in tumors, the citrate accumulation, and the ATP citrate lyase route leading to fatty acid synthesis and lipogenesis forming triglycerides, while OAA drives transaminations. This pathway is essential for tumor cells (see the addendum on polyamines).

In summary, it is like if the mechanism switching from neoglucogenesis to glycolysis was jammed in tumors, PK and PDH are at rest, like for neoglucogenesis, but citrate synthase is on. Thus, citric acid condensation pulls the glucose flux in the glycolytic direction, which needs NAD+, it will come from the pyruvate to lactate conversion by lactate dehydrogenase. The citrate condensation consumes acetyl-CoA and ketone bodies do not form. Citrate will support via ATP citrate lyase the synthesis of fatty, triglycerides and transaminations. The result of these metabolic changes is that tumors burn glucose while consuming muscle protein and lipid stores of the organism. In a normal physiological situation, one uses such stores to make glucose or ketone bodies but not for burning glucose!

Anoxia and anoxic tumors

Anoxic tissues draw their energy from a fermentation converting glucose into lactate; last steps are substrate phosphorylations that generate ATP at the phosphoglycerate kinase and pyruvate kinase reactions. This process is less efficient than the oxidative mechanism, which forms ATP at the expense of the energy potential of the proton gradient, flowing through the F1/Fo ATPase of the mitochondria.

One expects that hypoxic tissues will need more glucose for covering their energetic demand (Pasteur Effect). We have seen that the conversion of pyruvate into lactate, by-lactate dehydrogenase, provides the NAD+ for the glyceraldephosphate dehydrogenase step. The anoxic tissue releases lactate, building up an acid-lactate gradient around the tissue. In order to trigger back an oxidative metabolism, anoxic tissues require the help of red blood cells that will carry oxygen until the heart of the anoxic tissue. However, to do so, erythrocytes will have to penetrate deeper and deeper into the lactic acid gradient. Red blood cells do not have a mithochondria they have a glycolytic metabolism. When they penetrate into the lactic acid gradient, they will have more and more difficulty for releasing their lactate against an increasing external lactate concentration. This has an effect on up-stream glycolytic reaction products within the red blood cell; it is probable that lactate and pyruvate concentrations increase as well as 3Pglycerate, in the cytosol of the cell and one expects that more 1-3 DPG will isomerize into 2-3 DPG while penetrating in the lactate gradient. The 2-3 DPG is a negative allosteric regulator for oxygen binding to hemoglobin, its concentration increases, which releases more oxygen from hemoglobin. Hence, more and more oxygen reaches the anoxic tissue because 2-3 DPG increases in proportion to the external lactate. Moreover, since there is an acid gradient parallel to the lactate gradient, protons will dissociate oxy-hemoglobin (Bohr effect) releasing more and more oxygen from red blood cells that penetrates in the anoxic tissue. The oxygen delivered in proportion to the lactic acid gradient will re-start an oxidative metabolism in the anoxic tissue. Indeed, oxygen captures electrons and in a way, pulls the protons through the F1/F0 ATPase forming water. This process generates ATP in the mitochondria. The consummation of electrons and protons favors the conversion of NADH into NAD, the mitochondria NADH concentration decreases. Since NADH blocks the citric acid cycle at the citrate synthase step, the Krebs cycle will start turning supporting oxidative metabolism and the electron transport chain. The oxidative source of ATP formed at the expense of the NADH potential takes over, putting an end to the anoxic fermentation process.

In tumors, lactate release and hypoxia are frequent, but in this case, the pyruvate needed does not come via Pyruvate kinase, which is blocked, but via alanine transamination. The difference in the pyruvate sources between a normal anoxic situation and tumor anoxia may have several consequences on the lactate gradient and the penetration of red blood cells in the anoxic tissue. Moreover, the increased transamination and amine products, attracts on the site polynuclear cells (opsonization) they react as if the tissue had been invaded by some bacteria, forming hydrogen peroxide and peroxynitrite around the anoxic area. The consequence is that erythrocytes will have trouble for crossing the barrier without haemolysis. This will maintain the anoxic situation of the tumor. Studies by Solomides on peroxidases and the lysis of bacteria (http://lucadeparis.free.fr/infosweb/solomides.htm) led him to describe chemical mixtures that display a peroxidase activity, iodinated protein and ferric compounds hydrolyzing hydrogen peroxide. He believed they had anticancer properties, but could not convince. Presumably, ferric compounds **Fe3O4** [28] **lactoferrin** [29] and other compounds extracted from Crucifer vegetables, [30]

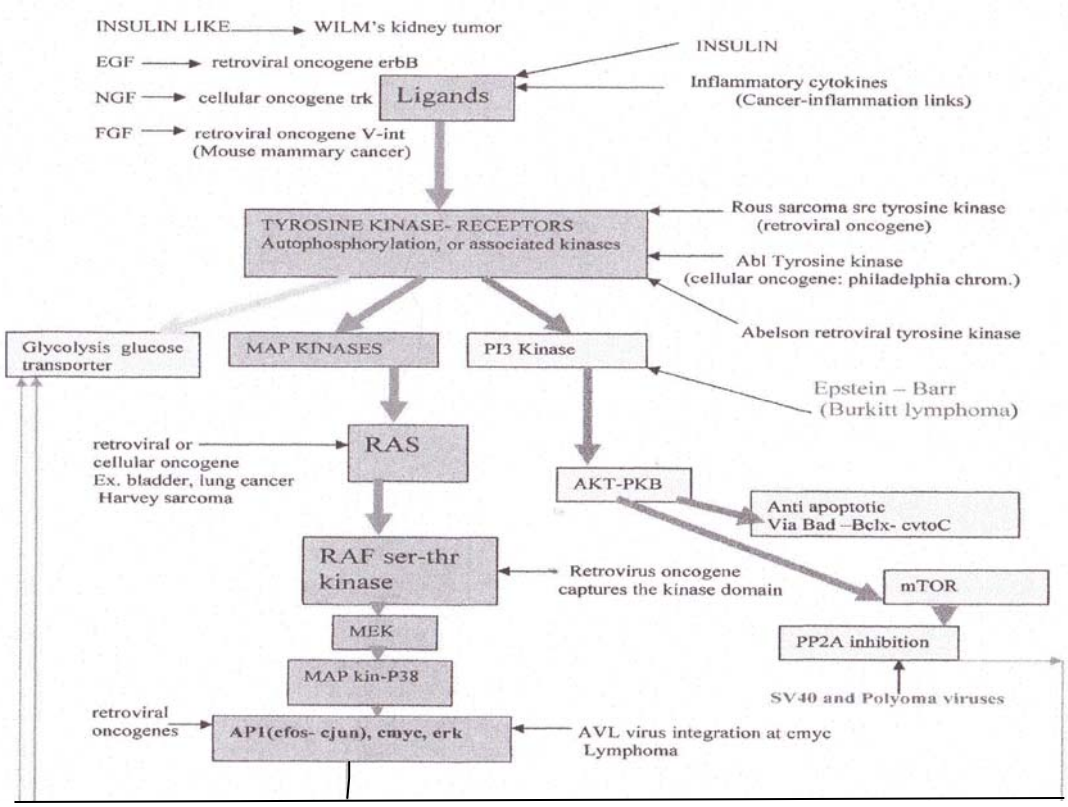

Fig. 2

Figure 2 Oncogene targets on signaling pathways

A retrovirus can capture a gene, from a host cell and transmit it to a new host. An upregulation of the gene product, now under viral control, may cause tumors. The captured gene has become a viral oncogene (v-oncogene), which derives from a normal host gene the proto-oncogene. The virus perturbs the expression of a cellular gene the proto-oncogene, by modifying its expression, or its regulation, or by transmitting a mutated form of the proto-oncogene captured from the previous host. Independently of any viral infection, a similar tumorigenic mechanism takes place, if the proto-oncogene is translocated in another chromosome; and transcribed under the control of stronger promoters. In this case, the proto-oncogene becomes an oncogene of cellular origin (c-oncogene). The third mode for converting a prot-oncogene into an oncogene occurs when a retrovirus simply inserts its strong promoters in front of the proto-oncogene enhancing its expression. The figure aims to show that retroviral oncogenes and cellular oncogenes disturb a major signaling pathway: the MAP kinases mitogenic pathways. At the ligand level we find tumors such Wilm's kidney cancer, resulting from an increased expression of insulin like growth factor; we have also the erbB or V-int-2 oncogenes expressing respectively NGF and FGF growth factor receptors. The receptor for these ligands activates the tyrosine kinase signals, similarly to the insulin receptor. The Rous sarcoma virus transmits a tyrosine kinase src that exerts its tyrosine kinase signal, leading to a chicken leukemia. Similarly, in murine leukemia, a virus captures and retransmits the tyrosine kinase abl. The tyrosine kinase step can also be enhanced if the tyrosine kinase abl is translocated and expressed with the bcr gene of chromosome 22, as a fusion protein (Philadelphia chromosome); in this case abl is a cellular oncogene. Furthur ahead, Ras exchanging protein for GTP/GDP, and then the Raf serine-threonine kinases proto-oncogenes are perturbed by corresponding oncogenes. Finally, at the level of transcription factors activated by MAP kinases, one finds cjun, cfos or cmyc; the latter, is for example, activated by the insertion of an avia leucosis virus. The figure indicates that retroviral attacks boost the MAP kinases similarly to inflammatory cytokins, or to insulin signals coupled to glucose transport and gycolysis.

A branch of the MAP kinase mitogenic pathway opens also the PI3 kinase pathway, PTEN phosphatase counteracts this effect, thus acting as a tumor suppressor. The scheme indicates that a DNA virus, the Epstein-Barr virus of infectious mononucleose, gives also the Burkitt lymphoma; the effect of the virus is to enhance PI3 kinase. Down stream, we show mTOR (the target of rapamycine, an immunosuppressor) mTOR, inhibits PP2A phosphatase, which is also a target for the simian SV40 and Polyoma viruses. Schematically, one may consider that the different steps of MAP kinase pathways are targets for retroviruses, while the different steps of PI3 kinase pathway are targets for DNA viruses. The viral-driven enhanced function of these pathways mimics the effects of their prolonged activation by their usual triggers.

act on tumors via a peroxidase effect. This allows erythrocytes to penetrate in the depth of the tumor without disruption; they might sense again the lactic acid gradient and provide the necessary oxygen for re-establishing an oxidative metabolism. The disruption of this "hydrogen peroxide shield" may be relevant in other pathologies as well. In Alzheimer's disease, amyloid plaques display a peroxidase activity that one would gain to study [31]. In Aids, lactoferrin seems to have antiviral effects [32].

Hypoxia is an essential issue to discuss. We suggest in other works that the transition from fetal to adult genes recalls a phylogenic adaptation of aquatic creatures to air and gravity. This transition concerns of course fetal /adult hemoglobin with different oxygen affinities, but many adult, more adapted proteins replace their fetal isoform: muscle proteins utrophine/dystrophine; enzymes such as the M2 PK [33] the M2 embryonic form being replaced by M1. Hypoxic conditions trigger back the expression of the fetal gene packet. The mechanism depends of a double switch since not all fetal genes are re-expressed. First, the histones have to be in an acetylated form opening the way to transcription factors, this depends either of HDAC inhibition or of HAT activation, and represents the main switch. Second, a more specific switch must be open, indicating the adult/ fetal gene couple concerned or more generally the isoform of a given gene that is more adapted to the specific situation. When the adult gene mutates, an unbound ligand may indeed indicate, the particular fetal copy gene to reactivate [34]. In anoxia, lactate and pyruvate are more difficult to release against the external gradient, leading to an increased up-stream glycolytic products, 3P glycerate or others. These products may then be the second signal controlling the specific switch for triggering the expression of fetal genes, such as fetal hemoglobin or the embryonic M2 PK; this takes place if histones (main switch) are in an acetylated form.

The "successful metabolism" of tumors, works at the detriment of the rest of the body, how did such a perversion occur? It would be difficult not to incriminate signaling routes exemplified by the "Insulin- tyrosine kinase receptor" signals, which regulate glucose entry, cell growth, anabolism and mitosis.

Signaling pathways affected by oncogenes

The discovery of P. Rous (1911), the Rous sarcoma virus, and the role of the src viral oncogene are a fundamental finding in the field of cancer, recalled in ref. [34]. In fact, the virus captures the viral oncogene from a previous host, [35] and encodes a protein src that is precisely a tyrosine kinase.

It is remarkable to notice that most retroviral oncogenes and cellular oncogenes, up-regulate one of the steps of signaling pathways exemplified by the tyrosine kinase receptor family such as the insulin receptor. Indeed, we know that insulin-receptor actions, phosphorylate receptor tyrosines, and after several steps serine-threonine kinases operate, supporting the MAP kinase -ERK signals, triggering mitosis. The figure 2 situates on a tyrosine kinase-signaling pathway the different viral or cellular oncogenes: at the ligand level, on the receptor or on the different steps of the MAP kinase cascade, see [15]. The other arm of this tyrosine receptor signal activates the PI3 kinase pathway, controlling via AKT-PKB glucose metabolism, anabolism and cell survival. The pathway is counteracted by PETEN phosphatase operating as a tumor suppressor. Well, DNA viruses such as the Epstein-Barr virus for mononucleose or Burkitt lymphoma enhance the PI3 kinase route [36]. Further ahead AKT-PKB controls mTOR, the target of an immune suppressor, rapamycin, which promotes anabolic processes, and the expression of ribosomal proteins. Well, mTOR inhibits again the phosphatase PP2A. This phosphatase is also the target of Polyoma and SV40 viruses [37-40], PP2A reacts with Polyoma middle and small T antigens and with small SV40, T antigen. These interactions would then cancel the effect of the PP2A serine-threonine phosphatase that acts as a brake over serine-threonine kinases, leading to an amplification of the "tyrosine kinase receptor" signal. Moreover, it is seems that astrocytomas associated to Tuberous Sclerosis regress after rapamycine the mTOR inhibitor. This genetic disease, affects the proteins harmatin and tuberin, the latter fails to inhibit a brain GTPase similar to RAS, which is a positive effector for mTOR, and rapamycin improves the situation [41]. Two different mTOR complexes have been identified, the mTORC1 associating Raptor and the mTORC2 associating Rictor and other proteins, rapamycine inhibits the mTORC1 complex. These complicated interactions were recently reviewed [42] showing the importance of the PI3 kinase signals counteracted by PETEN which is down regulated in cancer.

Oncogenes affect steps on signaling mechanisms that control cell mitosis, survival and the influx of glucose; triggering at different levels the metabolic perversion characterizing tumor cells. However, what is the scenario, which leads to this perverted metabolism?

The insulin tyrosine kinase receptor "poison" and the choline "antidote"

A crucial observation to reconsider

In a remarkable comment, Newberne [10] highlights interesting observations on the carcinogenicity of diethanolamine [11] showing that diethanolamine decreased choline derivatives and methyl donors in the liver, like does a choline deficient diet. Such conditions trigger tumors in mice, particularly in the B6C3F1 strain. Again,

the historical perspective recalled by Newberne's comment brings us back to insulin. Indeed, after the discovery of insulin in 1922, Banting and Best were able to keep alive for several months depancreatized dogs, treated with pure insulin. However, these dogs developed a fatty liver and died. Unlike pure insulin, the total pancreatic extract contained a substance that prevented fatty liver: a lipotropic substance identified later as being choline [9]. Like other lipotropes, (methionine, folate, B12) choline supports transmethylation reactions, a one carbon addition of methyles, to a variety of substrates, that would change their cellular fate, or action, after methylation. In the particular case concerned here, the removal of triglycerides from the liver, as very low-density lipoprotein particles (VLDL), requires the synthesis of lecithin, which might decrease if choline and methyl donors such as S-adenosyl methionine (SAM) are missing. Hence, a choline deficient diet decreases the removal of triglycerides from the liver; a fatty liver, and tumors, may eventually form. In some species, phosphatidyl ethanolamine may participate to the removal of triglycerides since a fatty liver is not a systematic observation for all species.

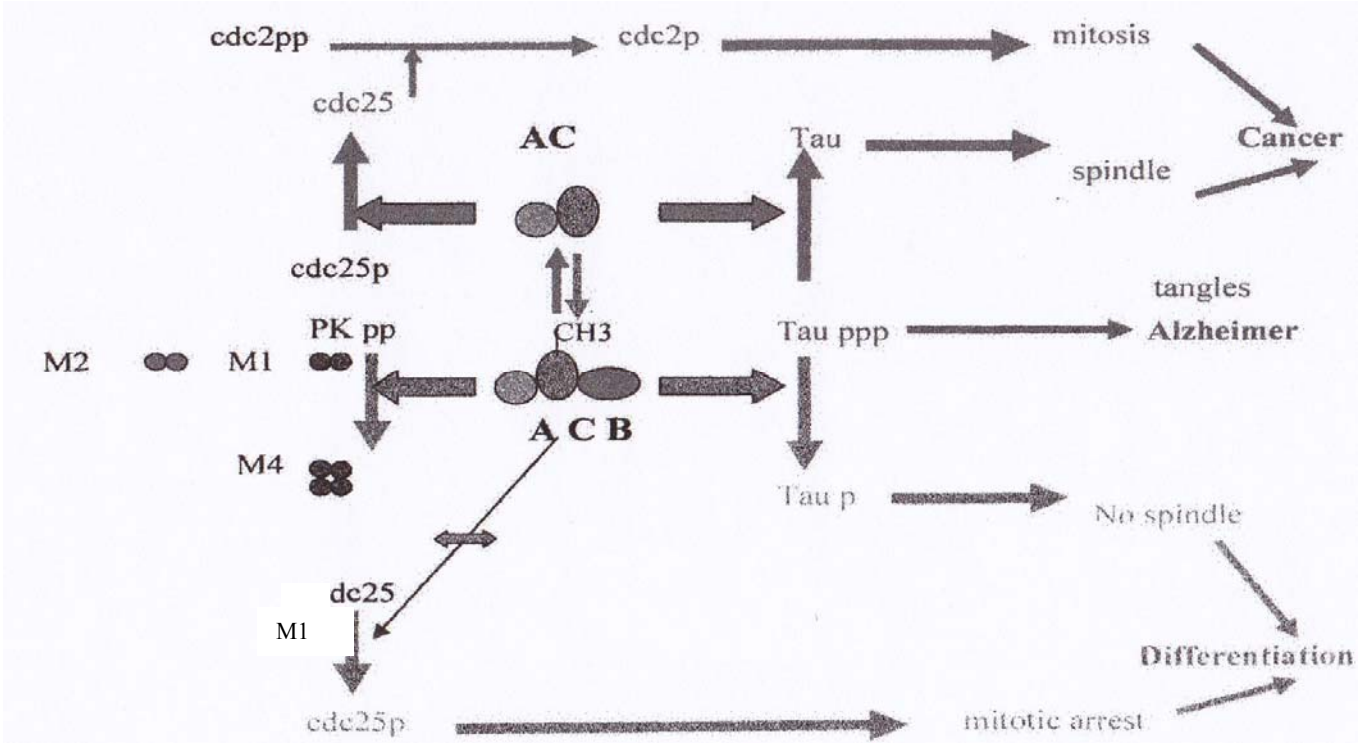

Fig. 3

Figure 3 PP2A phosphatase associations act on different phosphoproteins

*The PP2A, **AC** dimers have specific targets, they presumably dephosphorylate directly cdc25, Tau proteins, cell cycle proteins, which become activate; the spindle forms, and mitosis occurs. The PP2A dimers may have less affinity for pyruvate dehydrogenase or pyruvate kinase that remain phosphorylated and inactive, but would dephosphorylate diacylglycerol-3P, promoting the synthesis of triglycerides.*

*The PP2A trimer **ACB** forms when the catalytic subunit **C** gets a methyle. The trimeric phosphatase preserves cells from an abnormal Tau hyperphosphorylation (a feature of Alzheimer's disease). Another possible action of the trimeric phosphatase regulates the phosphorylation of Tau, inhibiting tubulin polymerization and the mitotic spindle. In addition, the trimers would not dephosphorylate cdc25p phosphatase inhibiting the cell cycle (cdc2pp), thereby inhibiting mitosis, and promoting differentiation. Moreover, the PP2A trimers would dephosphorylate and activate pyruvate dehydrogenase and pyruvate kinase M1, which forms an active tetramer. A methylation deficit does not assemble the trimeric PP2A. The M1 pyruvate kinase or its M2 isoform typical of tumor cells, are inactive forming the "bottleneck".*

*Another partner of **C** (not shown), is a protein **α4**, the **Cα4** phosphatase has antiapoptotic properties, favoring the immortal phenotype of tumor cells.*

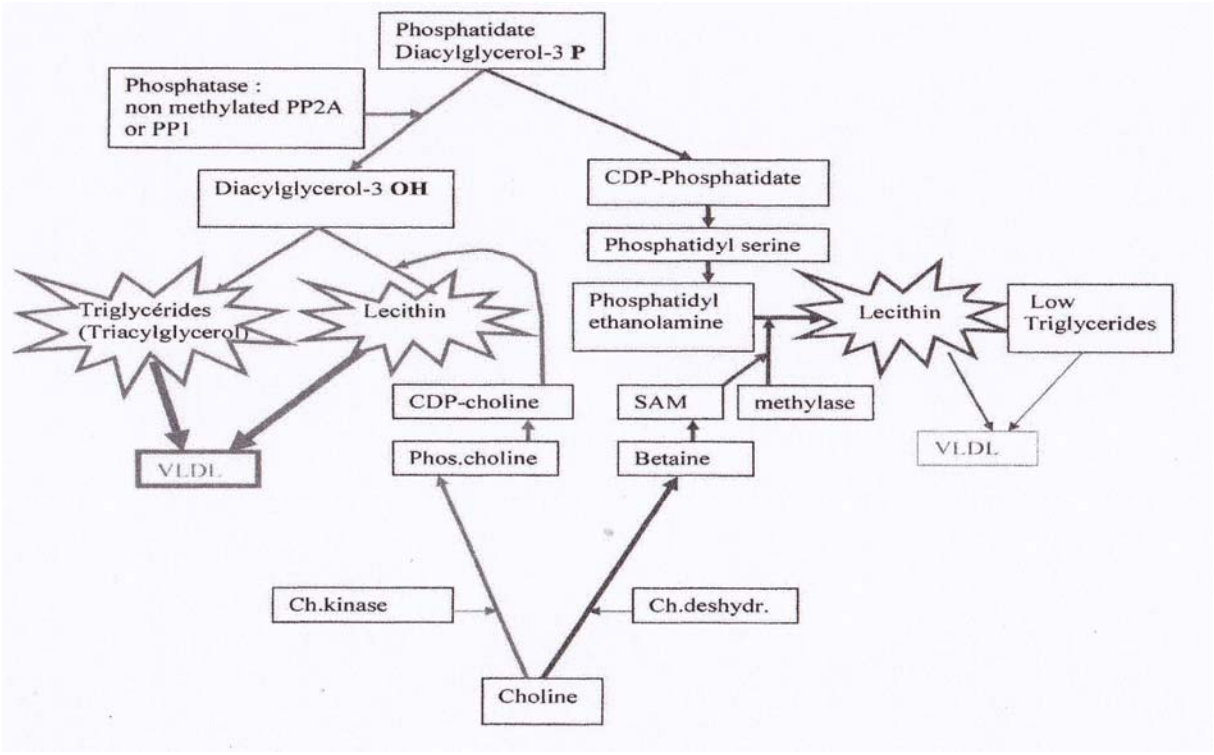

Fig. 4

Figure 4 Two lecithin synthesis pathways associated to different triglyceride contents

Left pathway*: a phosphatase (PP1 or Wp1 or PP2A non-methylated dimer) removes the Phosphate of Diacylglycerol-3P, allowing the acylation of the third carbone and the synthesis of triglycerides. In parallel the synthesis of lecithin via the choline kinase, phosphocholine and CDP-choline pathway takes place. Since triglycerides form in parallel, one needs more lecithin for removing them as VLDLs.*

Right pathway*: the phosphatase is not active, the third phosphorylated carbone cannot be acylated, and triglycerides do not form. Nevertheless, lecithin synthesis is still possible since diacylglycerol-3P (phosphatidate) gives CDP-phosphatidate then phosphatidylserine, the latter, is decarboxylated into phosphatidylethanolamine, which receives methyles via the choline dehydrogenase, S-adenosyl methionine (SAM) pathway. The lecithin synthesized by the methylation route has less triglyceride to remove.*

Tumors, use the choline kinase route rather than this methylation pathway, they will have more triglycerides to remove.

In sum, we have seen that pathways exemplified by the insulin- tyrosine kinase signaling pathway, which control anabolic processes, mitosis, growth and cell death, are at each step targets for oncogenes; we now find that insulin may also provoke fatty liver and cancer, when choline is not associated to insulin. We must now find how the lipotropic methyl donor controls the signaling pathway. ? We know that after the tyrosine kinase reaction, serine–threonine kinases take over along the signaling route. It is thus highly probable that serine-threonine phosphatases will counteract the kinases and limit the intensity of the insulin or insulin like signals. One of the phosphatase involved is PP2A, itself the target of DNA viral oncogenes (Polyoma or SV40 antigens react with PP2A subunits and cause tumors). We found the link between the PP2A phosphatase brake and choline in works on Alzheimer's disease [12]. Indeed, the catalytic subunit C of PP2A forms a dimer with a structural subunit A. When C methylates, the dimer A,C-CH3 recruits one of the various regulatory subunits B. The trimer ABC-CH3 then targets specific proteins that are dephosphorylated [13]. The figure 3 represents the methylation of PP2A and possible actions on specific proteins. In Alzheimer's disease, the poor methylation of PP2A is associated to an increase of homocystein in the blood [12]. The result of the PP2A methylation failure is a hyperphosphorylation of Tau protein forming tangles in the brain. Tau protein controls the tubulin polymerization, involved in axonal flow but also the mitotic spindle formation. It is thus possible that choline, via SAM, methylates PP2A, which is targeted toward the serine-threonine kinases that are counteracted along the insulin-signaling pathway. The choline dependent methylation of PP2A is the brake, the "antidote", which limits "the poison" resulting from an excess of insulin signaling. Moreover, it seems that cho-

line deficiency is involved in the M1 to M2 transition of PK isoenzymes [43].

Choline kinase rather than choline dehydrogenase in tumors

In fact, things are not so simple; several tumors display an increase of phosphorylcholine and choline kinase activity see for example [44, 45] this does not mean that other reactions involving choline methylations or lipotropic effects are not impaired. The increase of phosphorylcholine indicates that tumors accumulate triglycerides and use preferentially the "Kennedy" phosphorylcholine-CDP pathway for synthesizing lecithin. Evidently, the synthesis of triglycerides requires the dephosphorylation of diacylglycerol 3P into diacylglycerol in order to acylate the third carbon of triglycerides. The phosphatase involved could be PP1 or Wp1 that increase in cancer, or the non-methylated PP2A dimer. The diacylglycerol may thus form in parallel: triglycerides and lecithin. In order to give lecithin, diacylglycerol reacts with CDPcholine that comes from phosphorylcholine and CTP. This first pathway for lecithine synthesis, forms in parallel triglycerides, and lecithine, and operates in tumor cells because diacylglycerol is available after the phosphatase action (incidentally, recall that phorbol esters induce tumors via protein kinase C, activated by diacylglycerol). There is a second pathway for making lecithine. In this pathway, diacylglycerol-3P remains phosphorylated and thus, triglycerides cannot form. Nevertheless, lecithine synthesis is possible, since diacylglycerol - 3P can react with CDP, forming diacylglycerol-CDP. The latter, then reacts with serine or choline to give phosphatidyl serine (PS) or phosphatidyl choline (lecithin). PS can also be decarboxylated into phosphatidyl ethanolamine (PE), which then methylates, forming lecithin. This second pathway for synthetising lecithine, depends of methylations, and generates less triglyceride, facilitating the action of lipotropic compounds. This methylation dependent pathway is deficient in tumor cells that prefere the choline kinase route for making lecithine, which forms in parallel triglycerides, see the figure 4. Recall also that an increase of phosphorylcholine derivatives such as platelet activating factors (PAF), PAFacether, favor angiogenesis and the development of tumors, while PAF antagonists **(edelfosine, miltefosine)** inhibit the tumor [46] **.**

In addition, it is probable that membranes can become a source of choline, phospholipids, lecithin, when exogenous choline drops, this should maintain SAM levels, but would perturb the removal of triglycerides as VLDLs; since this process requires the synthesis rather than the hydrolysis of phospholipids. It is thus possible to have a properly methylated PP2A with choline coming from membranes, rather than from the diet; but again, if triglycerides are, elevated one may induce tumors. A hypothesis is that triglycerides presumably change the cellular compartmentation or properties of PP2A

Cellular distribution of PP2A

In fact, the negative regulation of Ras/MAP kinase signals mediated by PP2A phosphatase seems to be complex. The serine/threonine phosphatase does more than simply counteracting kinases; it binds to the intermediate Shc protein on the signaling cascade, which is inhibited [47]. The targeting of PP2A towards proteins of the signaling pathway depends of the assembly of the different holoenzymes. The carboxyl methylation of C-terminal leucine 309, of the catalytic C unit, permits to a dimer made of C and a structural unit A, to recruit one of the many regulatory units B, giving a great diversity of possible enzymes and effects. The different methylated ABC trimers would then find specific targets. It is consequently essential to have more information on methyl transferases and methyl esterases that control the assembly or disassembly of PP2A trimeric forms.

A specific carboxyl methyltransferase for PP2A [48] was purified and shown to be essential for normal progression through mitosis [49]. Similarly a specific methylesterase that demethylates PP2A was identified and purified, [50] it was found that the methyl esterase cancelled the action of PP2A, on signaling kinase that are indeed enhanced in glioma [51]. Evidently, the cellular localization of the methyl transferase (LCMT-1) and the phosphatase methyl esterase (PME-1) are crucial for controlling PP2A methylation and targeting. Apparently, LCMT-1 mainly localizes to the cytoplasm and not in the nucleus, where PME-1 is present; the latter, harbors a nuclear localization signal [52]. From these observations, one may suggest that PP2A gets its methyles in the cytoplasm and regulates the tyrosine-signaling pathway, attenuating its effects. A methylation deficit should then decrease the methylation of PP2A and boost the mitotic insulin like signals as discussed above for choline deficiency, steatosis and hepatoma. At the nucleus, where PME-1 is present, it will remove the methyl, from PP2A, favoring the formation of dimeric AC species that have different targets, presumably proteins involved in the cell cycle. It is interesting to quote here the structural mechanism associated to the demethylation of PP2A. The crystal structures of PME-1 alone or in complex with PP2A dimeric core was reported [53] PME-1 binds directly to the active site of PP2A and this rearranges the catalytic triad of PME-1 into an active conformation that should demethylate PP2A, but this also seems to evict a manganese required for the phosphatase activity. Hence, demethylation and inactivation would take place in parallel, blocking the phosphatase action on mitotic proteins. However, another player is here involved, the PTPA protein, which is a PP2A phosphatase activator. Apparently, this activator is a new type of cis/trans of prolyl isomerase, acting on Pro190 of the catalytic C unit isomerized in presence of Mg-ATP [54],

which would then cancel the inactivation mediated by PME-1. The demethylated phosphatase would thus become active again in the nucleus, on cell cycle proteins [55, 56] inducing mitosis. Unfortunately, the ligand of this new prolyl isomerase is still unknown. Moreover, we have to consider that other enzymes such as cytochrome P450 have also demethylation properties.

The hypothesis to consider is that triglycerides change the fate of methylated PP2A, by targeting it to the nucleus, there a methylesterase demethylates it; the phosphatase attacks new targets such as cell cycle proteins, inducing mitosis. Moreover, the phosphatase would render the nuclear membrane permeable to SAM the general methyl donor; promoters get methylated inducing epigenetic changes. The relative decrease of methylated PP2A in the cytosol, cancels the brake over the signaling kinases, but also favors the inactivation of PK and PDH, which remain phosphorylated, contributing to the metabolic perversion of tumor cells.

In order to prevent tumors, one should then favor the methylation route rather than the phosphorylation route for choline metabolism. This would decrease triglycerides, promote the methylation of PP2A and keep it in the cytosol, reestablishing the brake over signaling kinases. Moreover, PK, and PDH would become active after the phosphatase action. One would also gain to inhibit their kinases as recently done with dichloroacetate for PDH kinase [8]. The nuclear or cytosolic targeting of PP2A isoforms is a bold hypothesis inspired by several works [54-56] [52].

A brief survey of epigenetic changes in cancer

The entry of SAM in the nucleus stimulates methylases, allowing them to methylate CpG promoters, which recruit methyl-binding proteins (MBD) associated to HDAC (histone deacetylase). The latter, cuts off acetylated histone tails and "winds back" the DNA thread, silencing several genes such as PETEN phosphatase, which aggravates the elevated insulin signaling process (because PETEN hydrolyses phosphoinositides, and limits PI3 kinase effects associated to the tyrosine kinase receptor signal). In tumors, essential genes such as P53, a tumor suppressor, are silent. We shall see later that silencing IGFBP may also lead to uncontrolled mitosis. It is probable that a demethylase inhibitor gene is also silenced, activating a demethylase, which demethylates adjacent genes [16] allowing the recruitment over demethylated promoters of RXR receptor types, associated to histone acetylase HAT. The HAT-RXR complex acetylates histones, leading to an over-expression of other genes in adjacent regions, such as the hexokinase gene (the RXR receptors are similar to the steroid or thyroxin receptors, and to PPARs that play an essential role in cancer and inflammation). The histone acetylase recruited with NFkB, explains the link between the over expression of some genes in cancer and inflammation. In cancer, one finds at the level of DNA, hyper and hypo methylated regions. It is probable that an activated demethylase will not only demethylate promoters, but will also demethylate PP2A. Thus, the demethylated phosphatase may find new targets, such as cdc2 cdc25, cyclin dependent kinases, and other proteins controlling the cell cycle, or the RB protein, keeping mitosis activated.
We have discussed above the epigenetic changes induced by hypoxia, the genetic transition that opens the fetal gene cassette. Cells grow, multiply and their apoptotic mechanism is blocked. The tumor develops, burns glucose, releases lactate, and is fed by lipolysis and proteolysis taken from adipocytes and muscles. The triglycerides that form, change the cellular distribution of methylated PP2A, sustaining a dysmethylation process associated to new genetic programs that favor the development of the tumor.

Dividing stem cells and sterile differentiated cells: asymmetrical mitosis

Stem cells, early observations

André Gernez was first to explain diseases such as cancer or neurodegenerations through a theory of mitosis and differentiation that challenged prevailing ideas. He indeed considered that in a tissue, only limited populations of cells were able to divide. He even compares a tissue to "a colony of bees, in which, only the Queen ensures reproduction, while most of the other bees are sterile workers". This theory contradicted the current anthropomorphic view considering that cells grow, divide and die. Gernez was the first to apply to pathology the stem cell concept that was fortunately published it in his books [57-59]. This meant that a dividing stem cell had to different daughters, one gave back a stem cell inheriting the mitotic capability, while the other sterile daughter, was programmed to differentiate. Gernez applied to cancer and neurodegenerations the stem cell concept. His contributions were recently recalled see ref [60-61]. However, like many new ideas that came too early, Gernez's theories did not prevail, delaying for many years an explanation of mechanisms leading to cancer or neurodegenerative pathologies.

In the case of cancer, it is precisely an abnormal situation where the two daughter cells inherit the mitotic capability

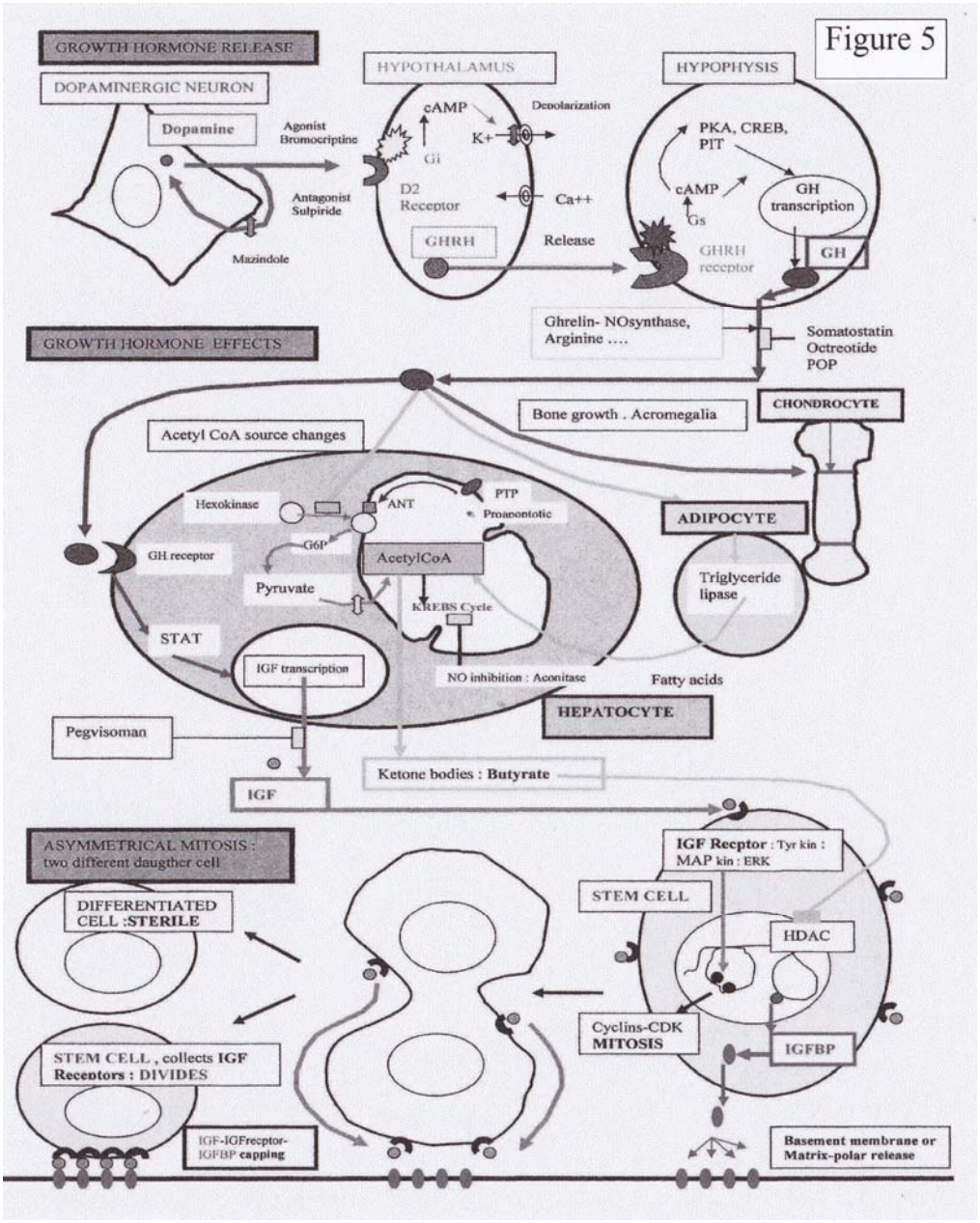

Figure 5 *Growth* ***Hormone- IGFeffects.***

Top panel*: it shows a dopaminergic neuron acting on hypothalamic neurons that release GHRH (Growth Hormone Releasing Hormone). Dopamine stimulates hypothalamic D2 receptors coupled to Gi. Agonists (bromocriptine) increase GHRH release, antagonists (sulpiride) decrease GHRH release. By inhibiting dopamine uptake, mazindole depresses the dopaminergic neuron, which reduces hypothalamic GHRH release. The Gi effect decreases cAMP, closes K+ channels, a subsequent depolarization triggers a Ca++ entry, which elicits the release of GHRH. At the anterior hypophysis somatotroph cells, activated by GHRH, release Growth Hormone GH (or somatotropin). GHRH acts upon a receptor coupled to Gs. Here, the increase of cAMP activates PKA, CREB, PIT and the transcription of GH, increasing the content and release of GH. The somatotroph cell bares also D2 receptors (not represented) these are coupled to Gi, and their activation by bromocriptine decreases cAMP, which attenuates the effect of GHRH; the overall effect of bromocriptine is a decrease of GH release, in spite of the fact that bromocriptine increases GHRH release at the hypothalamic level. Other triggers of GH release are Ghrelin released by the stomach, arginine, NO. Other hormones, somatostatin and analogous peptides (ocreotide) inhibit GH release, compounds such as paraoxypropiophenone POP, are also GH inhibitors.*

Middle panel*: shows the different effects of GH: first, the classical action on chondrocytes and bone growth in childhood. The absence of GH gives dwarfs, an excess of GH in adults leads to Acromegalia. Second, the metabolic action in hepatocytes,*

there is a change in the source of acetyl-CoA, operated on one hand, by the activation of a triglyceride lipase in adipocytes, which provides fatty acids for β oxidation, and on the other hand by the inhibition of the glycolytic source of acetyl-CoA, resulting presumably from an inhibition of hexokinase- ANT interactions. The PTP transition pore no longer excluded by hexokinase may then join the site and apoptosis may occur in case there is a fault in mitosis. The lipidic source of Acetyl-CoA supports the production of ketone bodies and butyrate by hepatocytes. The third and essential action of GH on hepatocyte is to trigger the synthesis of the Insulin like Growth factor (IGF) via GH receptors; they form dimmers that act via STAT on IGF transcription, an effect inhibited by pegvisoman.

***Lower panel**: it illustrates asymmetrical mitosis; the mitotic trigger IGF acts on a stem cell. The dividing cell expresses and releases IGFBP a protein that binds IGF. This might be modulated by butyrate since butyrate inhibits histone deacetylase (HDAC) favoring the expression of genes. The hypothesis is that after release, IGFBP forms patches on the basement membrane, at a site of polar release. The IGFBP Patch provokes the capping of the IGF receptors (IGFR) bound to IGF. Hence, only the daughter cell adjacent to the patch inherits the complex and IGFR, it will thus become a stem cell able to divide. The other daughter cell devoid of IGFR will be sterile and differentiates. In this type of mitosis, one stem cell replaces one stem cell, explaining the constant mass of organs. If this capping system is perturbed (no IGFBP) the two daughter cells become stem cells, there is a geometrical increase of their number like in tumors. Drugs such as apigenin and others that increase IGFBP would limit IGF effects.*

of a dividing cell, explaining the geometric increase of the tumor mass. In contrast, in a normal tissue, one of the daughter cells replaces the mother stem cell; while the other differentiates, keeping constant the organ mass.

In degenerative and neurodegenerative diseases, the stem cell concept changes the therapeutic possibilities. In a brain altered by Alzheimer's disease, plaques and tangles impair major brain functions. If one spares stem cells, they may overcome the situation by replacing dead neurons at least in some areas. In Parkinson's disease, dopaminergic therapies tend to boost the activity of residual cells with L dopa, the dopamine precursor; this improves the situation for a while, but does not spare stem cells. Gernez considers that one should inhibit the striatum, which has an increased activity resulting from the loss of dopaminergic neurons. This would restore the striatal control over the pallidum and the muscle tone. As for stem cells, it is a capital to spare. Stem cells, would divide about 70 times, fixing a time limit to life. Thus, therapies that spare stem cells are to be encouraged. In the future, grafting new stem cells may become possible. Indeed, differentiated cells can be convert into pluripotent cells that resemble embryonic stem cells, using a few transcription factors transiently expressed, after transfection with adequate plasmids, this opens new medical possibilities, but much work is still necessary [62-65].

It seems essential to understand the signals that control the asymmetrical mitosis of a stem cell, leading to two different daughters: a new stem cell and a sterile one. This is a key for cancer prevention. We have seen above how tyrosine kinase receptors exemplified by the insulin receptor, induced via RAS GTPase and serine threonine kinases, anabolism and mitosis. The so-called MAP kinase ERK pathway and PI3 kinase routes mediate these actions; in addition to PLC- DAG- IP3 effects. Most tyrosine- kinase receptors like the insulin or ephrin receptors and others activate these pathways controlling mitosis and anabolism. Hence, all steps of such pathways are potential targets for viral or cellular oncogenes, and inhibitors of the tyrosine kinase signals led to interesting anti-cancer drugs. We have discussed metabolic controls of carbohydrate metabolism; and their implication in tumors. Recall that the methylation of a phosphatase PP2A brings it over the tyrosine-kinase receptor pathway, and that it counteracts the serine – threonine kinases signals, acting in a way like many new anti cancer drugs. We have supposed that choline the lipotropic vitamin and methyl donor methylated PP2A, preserving from an excess of insulin signaling.

Insulin and IGF

Insulin release responds to an elevated glycemia. Glucose elicits an increase of ATP in the βcells of the pancreas, ATP closes K+channels; the resulting cell depolarization triggers a calcium entry and the release of insulin, probably associated to choline or choline derivatives. However, "tyrosine kinase receptor" activation is not only the result of an insulin action; many similar receptors exist and respond as well to other ligands or growth factors. We shall now give a special attention to the Insulin-like growth factors (IGF) initially named somatomedine or sulfatation factor because it increases in parallel, the incorporation of sulfate in cartilage. IGF, is regulated by specific controls (figure 5), more than anabolism, mitosis is the finality. Mitosis is normal as long as one daughter cell inherits the mitotic capability, while the other sterile daughter differentiates; it is an asymmetrical mitosis. We shall now discuss IGF controls and propose a mitotic mechanism attributing to a protein binding IGF abbreviated (IGFBP), the control of asymmetrical mitosis. IGF secretion from the liver, results from growth hormone (GH) action. While GH, is a secretion of the hypophysis, the anterior pituitary gland. In this way, GH controls the development of the organism supporting the mitosis of stem cells from the embryo until the adult. We know that its absence leads to dwarfs, its excess to gigantism. In the adult, GH secretion decreases but is still found, presuma-

bly controlling the remaining stem cell population involved in the replacement of dead cells. Each stem cell has limited number of mitosis, probably related to the length of chromosomal telomeric ends. The total population of stem cells, and their mitotic cycles, controls a maximum lifetime. In the adult, an excess of GH leads to a disease: Acromegalia, showing hypertrophy of bones

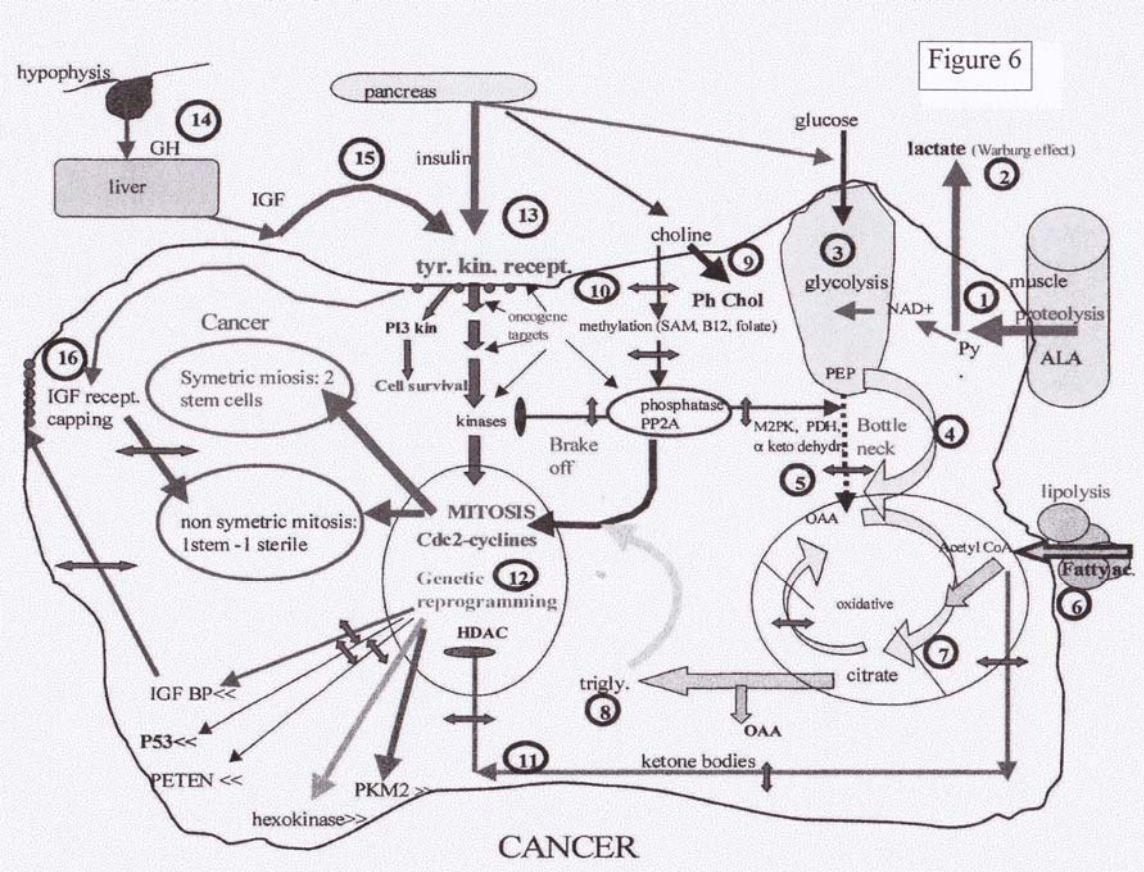

Figure 6 Pharmacological, targets in tumor cells.

The figure gives a synthetic view of metabolism, signaling and genetic changes in tumor cells; it also proposes a mechanism for their symmetrical mitosis. A stimulation of tyrosine kinase receptors exemplified by the insulin receptor, triggers the MAP serine-threonine kinase signals that elicit mitosis, when they are not counteracted by a serine-threonine phosphatase (PP2A brake) controlled by methylation. When the methylation pathway is deficient, the choline kinase route increases. The figure shows the enhanced glycolysis interrupted by the PK bottleneck. Above the neck, glycolysis operates in association to muscle proteolysis; alanine transamination feeds lactate dehydrogenase, generating NAD+, and the lactate released. Below the neck Citrate, condensation operates at the expense of lipolysis. Triglycerides increase, ketone bodies decrease. Epigenetic effects turn off genes and up-regulates other genes.

In parallel GH –IGF- IGFBP pathways are supposed to control the distribution of the IGF receptors in the mitotic daughter cells a hypothesis discussed in figure 5.

In order to develop a possible correction of this perverted metabolism, we identify here several targets that we ***number*** *on the figure; each target covers several enzymes in the pathway. A list of compounds acting on these numbered targets is in the text.*

and organs. It is highly probable that an excess of GH stimulates, via IGF, the mitosis of stem cells until an accidental abnormal mitosis occurs, which is the starting point of tumors. Considering that Acromegalia favored cancer in contrast to hypopituitarism, Gernez proposed to decrease cancer risk by a selective irradiation of GH releasing cells of the hypophysis (Gamma Knife). It is probably easier to inhibit with selective drugs GH release or neutralize its effects. Moreover, Gernez also noticed that mental patients treated with chloral (this was before largactil) had less cancer. We also know that dopaminergic neurons trigger GH release; thus, in Parkinson's disease, a decrease of GH release seems associated to a re duced cancer risk [66]. In the same line, one should note that GH release depends of other hormones such as growth hormone releasing hormone (GHRH), or of the Ghrelin/leptine ratio, (ie the hunger /saciety ratio) establishing a link between cancer risk and obesity.

Growth hormone-IGF metabolic actions, the control of asymmetrical mitosis

We have discussed the link between an efficient glycolysis and the survival of cells. We have seen that it was necessary to maintain the insulin action within limits, preserving the anabolic effects while inhibiting mitosis.
Presumably, via a choline mediated methylation of PP2A, targeted over the signaling tyrosine kinase pathway. We also discussed the role of accumulated triglycerides that perturb the phosphatase or target it to the nucleus.
The insulin action and resulting glycolysis, provides acetyl-CoA for the citric acid – Krebs cycle via pyruvate dehydrogenase. If instead of insulin, IGF-GH is at work, the acetyl-CoA source will change, since GH stimulates a triglyceride lipase in adipocytes, increasing the fatty acid source of acetyl-CoA. The latter, will essentially come from the β oxidation of acylCoA, which enters in mitochondria via the carnitine transporter. In parallel, GH will probably close the glycolytic source of acetyl-CoA, presumably by inhibiting the hexokinase interaction with the mitochondrial ANT site. The hexokinase becomes more distant from the source of ATP, which renders apoptosis possible, because the PTP transition pore now joins the site. This is essential, because the mitogenic GH – IGF action must be safely associated to a possible apoptosis, in case a fault occurs in mitosis. On the contrary, the hexokinase- ANT interaction increases after GH removal, as suggested by the old and classical experiment of Houssay. He showed that diabetes (after pancreatectomie) improves after hypophysectomie. One may then suppose that the decrease of GH, increases the influx of glucose in the cell, because the hexokinase – ANT interaction becomes stronger, which pulls in the glucose flux and decreases glycemia.

We know that many triggers lead to GH release, among them: the stomach hormone Ghrelin (acting via - NO synthase- arginine), or deep sleep, or hypoglycemia induced by insulin. GH will mobilize the fatty acid source of acetyl-CoA from adipocytes supporting the formation of ketone bodies and butyrate. Indeed, Arginine- NO enhances GH release, but also interferes with citrate condensation, since NO inhibits aconitase, favoring the ketogenic route but also the ATP citrate lyase pathway. Butyrate is a natural histone deacetylase (HDAC) inhibitor; preserving histone acetylation and the transcription of several genes implicated in mitosis. We also think that butyrate activates the expression of essential genes that support the transcription, and release of IGFBP, which binds to IGF with high affinity to control its effects [18]. In parallel, GH hormone induces in the liver, the synthesis and release of IGF. The latter activates IGF- tyrosine kinase receptors (IGFR), triggering the MAP kinase ERK mitogenic signal. The surface distribution of IGF- IGFR should then determine if a cell is sterile or endowed with a mitotic potential. It is then necessary to find factors that regulate the distribution IGF- IGF receptors between the two daughter cells. We propose that this distribution depends of IGFBP. The increased butyrate resulting from the metabolic action of GH on liver cells induces the transcription of many genes (often expressed in juvenile cells) among them, IGFBP. Well suppose that a cell starting mitosis secretes IGFBP at one pole, and that IGFBP binds to the extracellular matrix, forming patches of IGFBP. The patch will attract the IGF- IGFR complex by a capping process. The daughter cell that inherits the IGFR receptor patch becomes a stem cell, while the cell poor in IGFR will be sterile and follow the differentiation program (Figure 5).
This does not mean that the sterile daughter cell, at rest in the Go phase, cannot exit and divide again; this would require a trigger for reactivating the IGFR receptor gene in order to build up again the membrane concentration of IGFR.

In summary, GH elicits the production of IGF by the liver, the latter triggers mitosis. GH also mobilizes fatty acids, changing the source of acetyl-CoA, which favors the formation and release of butyrate by the liver. Butyrate inhibits HDAC in the dividing cell and activates the expression of IGFBP. A capping effect mediated by IGFBP leads to an asymmetrical mitosis giving: a daughter stem cell inheriting the IGFR patch and a second sterile daughter, poor in IGFR. In cancer, the GH- IGF mitotic trigger is in excess and IGFBP reduced, the two daughter cells inherit IGFR and the mitotic potential. see figure 5

The other effect of GH is on chondrocytes controlling the growth of bones (figure 5) we only mention it here. The respective role, of insulin, and IGF, in cancer is in the discussion part of ref [17]. Here we propose a possible hypothetical mechanism, which establishes a link between asymmetrical mitosis and the IGF signals. We do not exclude other possible mechanisms supporting asymmetrical mitosis related to centriole duplication, controlling the spindle axis and distribution. Such mechanisms do control the volume of post-mitotic embryonic daughter cells, which depends of so-called PAR proteins differentially distributed at cellular poles.

Summary of cancer cell features: numbering possible therapeutic targets

The figure 6 gives a synthetic view of metabolism, signaling and genetic changes in tumor cells, and suggests a possible mechanism for their symmetrical mitosis. The **numbers** indicate targets for a pharmacological attack of

tumors, with compounds listed in next section. The figure summarizes all the points discussed above in detail. The stimulation of tyrosine kinase receptors exemplified by the insulin receptor triggers the MAP serine-threonine kinase signals that elicit mitosis, when a serine-threonine phosphatase (PP2A brake) does not counteract the kinases. In parallel, the tyrosine kinase receptor activates the phosphatidyl inositol –3 kinase route (PI3 kinase), which controls via protein kinase B (PKB) anabolism and cell survival, this pathway not represented in the figure 6 is counteracted by a phosphatase (PETEN). The tyrosine-kinase receptor activation also leads to a stimulation of proteine kinase C (PKC), via phospholipases that increase diacylglycerol (DAG) and inositol 3 phosphates (IP3) not represented. The latter mobilizes internal calcium stores, erties of a phosphatase PP2A are here essential. When the catalytic subunit of this phosphatase is in the methyl form, (CH3- PP2A) a trimeric enzyme assembles, and targeted to specific substrates. In this particular case, it attenuates the MAP kinase route, linking the methylation capability of cells and the brake, which attenuates these mitogenic signals. Methylation depends of several factors, vitamin B12, folate, choline, S-adenosyl methionine (SAM) the general methyl donor. The latter methylates PP2A and thus supports its regulatory function on mitosis. The supply of choline a methyl donor, but also a lipotropic factor, does protect the liver from steatosis and cancer. In tumors, choline does not follow the methylation route starting with choline dehydrogenase but feeds choline kinase, forming lecithine and in parallel triglycerides. The poor methylation of PP2A has other consequences; it fails to dephosphorylate pyruvate kinase (PK) and pyruvate dehydrogenase (PDH) that are blocked. In tumors, the M2 PK remains phosphorylated and inactive; there is a bottleneck at the junction between glycolysis and oxidative metabolism. The tumor cell overcomes the bottleneck, at the detriment of the organism, muscle proteins are proteolysed providing alanine (Ala), which is transaminated by ALAT to form pyruvate, the latter is converted to lactate by lactatedehydrogenase (LDH), lactate is released by the tumor (Warburg effect), while the NAD+ formed is provided to glyceraldehyde dehydrogenase allowing glycolysis. Since PDH is blocked, pyruvate is no longer a source of acetyl-CoA, which will come from lipolysis and fatty acids β oxidation. The Oxalate (OAA) necessary for the citrate condensation with acetyl-CoA, comes from phosphoenol pyruvate (PEP) accumulated above the PK bottleneck, via PEPcarboxykinase. The citrate condensation is rather active in tumor cells, presumably because its NADH inhibitor is low. Moreover, the conversion of acetyl-CoA into citrate is associated to a poor formation of ketone bodies (butyrate) they fail to stimulate pyruvate carboxylase, which might have provided OAA, leaving more pyruvate to LDH. In a normal situation lipid and muscle, proteins mobilize for making glucose and ketone bodies in order to feed tissues, after starving for example, but tumors mobilize such stores for burning glucose! This is a consequence of the bottleneck described. In addition, the elevated citrate condensation provides with substrate the enzyme ATP citrate lyase, forming acetyl-CoA in the cytosol, which starts the synthesis of fatty acids. They react with diacyl glycerol (DAG) to form triglycerides (tri gly) that accumulate, while the OAA formed in parallel in The accumulation of triglycerides influences the intracellular localization of CH3- PP2A and SAM that move to the nucleus, inducing the epigenetic changes. SAM methylates CpG promoters, which recruits MBD (methyl binding proteins) and HDAC, the latter deacetylates histones and silences genes, particularly in tumors, since butyrate decreases and stops inhibiting HDAC. The PETEN gene is silenced, which strengthens PI3 kinase effects, anabolism and cell survival, these effects are mediated by PKB, and the activation of the antiapoptotic protein BCL 2. The figure 6 indicates that among the genes that silence, one finds PETEN, the tumor suppressor P53 and presumably many others. An inhibitor of methylesterase is probably off as well. This induces the demethylation other adjacent DNA regions that will recruit RXR receptor types in association with histone acetylase (HAT). Histone acetylation boosts the expression of other genes such as the hexokinase (Hex) or genes encoding proteins supporting the cell cycle cyclins (cyclin dependent kinases, and phosphatases such as cdc2, cdk, and cdc25). The hexokinase expression will pull the influx of glucose by converting it in glucose 6P, and inhibit the apoptotic triggers (not shown). Recall that an up regulation of the BCL2 protein has similar effects. Thus, the tumor cell resists to apoptosis.

The cytochrome P450 demethylase may also be involved, and probably demethylates PP2A, like its specific esterase found in the nucleus, the phosphatase will then recognize new targets such as cdc25 or cdc2 that are dephosphorylated, which activates their catalytic effect and mitosis.

helping the exocytotic incorporation of the glucose transporter in the membrane, which increases the influx of glucose.
The routes that become active after tyrosine kinase receptors stimulation are at each step the targets of most oncogenes. An up regulation of these signals may transform a normal cell into a tumor cell, if brakes keeping these signals within their normal limits do not operate. The propthe cytosol drives the transaminases and changes amine metabolism in tumors (see addendum).
An elevated citrate condensation, which lowers ketone bodies, has epigenetic consequences because butyrate is a histone deacetylase (HDAC) inhibitor, this leads to a genetic reprogramming as indicated in the figure 6.

When the insulin receptor elicits the mitotic process, because the methylated PP2A brake does not counteract it, the two daughter cells inherit these receptors in their membrane and inherit in parallel a mitotic potential, it is thus preferable to keep these receptors regulated, since they are presumably more involved in anabolic processes. The figure indicates that choline is here essential, acting as a methylating agent, a lipotropic factor and possibly as ligand for G-coupled receptors. The figure 6 indicates that Growth hormone (GH) elicits, via Insulin-like growth factor (IGF) secreted by the liver, the activation of similar tyrosine kinase receptors. Mitosis takes place in a different way; only one daughter cell inherits the receptors and the mitotic capability, the other cell being sterile and committed to differentiate. The hypothetical mechanism explaining this non-symmetrical mitosis results from the properties of an IGF binding protein (IGFBP), which has a high affinity for IGF. Presumably, released IGFB binds locally to the basal membrane or extra-cellular matrix, forming a patch with the IGF- IGF receptor complex. The latter, gathers in a single daughter cell, which inherits the receptor and a mitotic capability as discussed above. In tumors, a shortage of IGFBP may disperse the patch and the receptor complex in the two daughter cells that become both mitotic cells, leading to a geometric increase of the tumor mass.

A combination of drugs acting on tumor cell targets

The only way for counteracting the "successful metabolism of tumor cells" is an attack by a combination of drugs. The numbers in figure 6 represent different pharmacological targets that we list; each target covers several enzymes in the pathways. **Target 1**, we have seen that the PK "bottleneck" renders the tumor avid for pyruvate, coming from muscle proteolysis and alanine transamination, this process has several steps to attenuate with inhibitors of alanine transaminase (ALAT) **aminooxacetic acid** or with **hydroxylamine** see ref [67] or **pralidoxime.** Compounds such **Br-alanine, D-alanine, β- chloro-L – alanine** [68] may also inhibit this pathway. We recall that a group of amino acids (α. amino dicarboxylic amino acids) referred as gliotoxins, disrupt in astrocytes the metabolism of alanine and glutathion production [69]. These compounds, **L-α-aminoadipate, L-serine-O-sulphate, D-aspartate, L-cysteate** affect like **L-anti-endomethanopyrolidine dicarboxylate** the sodium-dependent transport of amino acids and may then act on target 1. The conversion of pyruvate to lactate (Warburg effect) represents **target 2** , NAD+ is needed for glycolysis and the tumor is particularly dependent on lactate dehydrogenase (LDH), this enzyme can be inhibited with **Br pyruvate** [70] or with compounds such as **gossypol** [71,72]. The glycolytic pathway itself figures in **target 3**; several compounds may decrease glycolysis, **mannoheptulose** [73] or **2-deoxyglucose**. Bait peptides (hexokinase minus glucokinase) that would displace hexokinase from the mitochondrial site, or **lonidamine** [74] deserve a try. The polyol route should also be attenuated there are drugs that have been developed: **epalrestat** and **raniresta** [75]. Flavonoids and plant polyphenols are also interesting since they inhibit aldose reductase: Cinnamomum cassia contains **cinnamaldehyde**, which carries the effect; **quercitin** is also a potent inhibitor. Many similar compounds act on **target 3**. One would also gain to study the "Neuberg effect" on glycolysis and fermentation, recall that bisulfite deviate glycolysis toward the formation of glycerol because bisulfite forms addition compounds with pyruvate and aldehyde. The effect of bisulfite and glycerol on tumor glycolysis deserves some studies. In addition, bisulfite has demethylating actions on methylated DNA. Moreover, it might be useful to cancel the pyruvate carboxylase inhibition of tumors and divert pyruvate from LDH, ketone bodies **(3-hydroxybutyrate), amonaps,** stimulate pyruvate carboxylase [76]. In tumors, PEP carboxykinase is a source of oxaloacetate, condensed with acetyl-CoA, this step represents **target 4**; one may inhibit PEP carboxykinase with **phosphoenol-3-bromopyruvate Br–PEP** [77] or **chloro phosphoenol pyruvate Cl-PEP** [78] or even, **β sulphopyruvate,** one may try to inhibit selectively the mitochondrial enzyme with a **carnityl** ester of **Cl-PEP or Br-PEP.** Other compounds inhibit this enzyme: **3-mercaptopicolinic acid** [79] or **mebendazole, albendazole,** [80] or substituted **dibenzylxanthines,** [81] acting on the GTP site of the enzyme, or even **hydrazine** [82] the latter should be comparable to **cryogenine, or phenelzine**. The requirement of CO2 by PEP carboxykinase might be perturbed, or changed with avidin, or with an inhibitor of carbonic anhydrase (**acetazolamide)** [83] or with **vitamin K** [84]. **Target 5** is an essential one, it concerns PK but also PDH that are inactivated by phosphorylation. In tumors, the M2 embryonic PK is a typical feature, essential works of Eigenbrodt [85] show that the inactive enzyme forms a bottleneck, between glycolysis and the entry in the citric acid cycle. One may try to help the dephosphorylation of PK; via activators of PP2A phosphatase such as **dihydroxyphenylethanol** [86], this should also activate PDH. **Fructose 1-6 bis P**, also stimulates M2 PK. Recent works [14, 20-21] establish a link between an excess of tyrosine kinase signaling and the inactivation of PK, M2. The latter, binds phospho tyrosine, and this cancels the fructose1-6 bis P activation of PK. Experiments show that **Polyethylene glycol (8000)** stimulates PK [87] and represses colon cancer [88]. For stimulating PDH, vitamin B1 may also help. Alternatively, one might inhibit the kinases for these enzymes as done for PDH, with **dichloroacetate** [8]. A comparison of dichloroacetate and **2-chloropropionate** [89] shows that the latter is an exclusive PDH activator, whereas a dichloroacetate metabolite

(oxalate) inhibits also pyruvate carboxylase, which is not a desired effect. Other compounds acting on this target include **amirinone** [90] **or ranolazine** [7] or **lipoic acid** [91]; it would be interesting to study the effect of **dimercaprol**, British anti Lewisite (BAL). We have also seen that α ketoglutarate dehydrogenase closely resembles PDH, and might respond to similar controls; its activation would increase the production of NADH turning off the abnormal citrate condensation of tumors. Opening PK and PDH means that one switches, to a metabolism compatible with an active citrate synthase, which is abnormally on. **Target 6** represents the pathways that provide acyl – CoA and acetyl- CoA to feed the condensation reaction. It is preferable not to inhibit carnitine acetyltransferase because a decrease of the acyl-CoA supply to mitochondria, would not only inhibit the condensation reaction, but also the production of acetyl CoA and ketone bodies that we want to increase for inhibiting HDAC, and stimulate pyruvate carboxylase. It is thus preferable to control this target 6 by limiting the oxaloacetate coming from PEP. Moreover, an inhibition of carnitine transporter acylation would accumulate fatty acids at the mitochondrial door in the cytosol. In addition, we find in the literature, a case of fatal hypoglycemia with levofloxacin a carnitine acetyltransferase inhibitor [92]. One may try on animal models, to inhibit this step with **oxfenicine** [93]. However, it seems better to reactivate the PDH source of acetyl-CoA rather than blocking the acyl carnitine source of acetyl-CoA. The fatty acid supply from adipocytes could be diminished using **niacine** associated to **statins**, favoring the increase of HDLs [94]. Other compounds developed for fighting atherosclerosis (fibrates) would not be an option; they certainly decrease triglycerides in the blood but impair the methylation of phosphatidylethanolamine [95]. It is perhaps more appropriate to act downstream directly on citrate synthase, which represents **target 7**, citrate synthase is inhibited by adenine nucleotides, ATP, **suramin** or **fluoro acetyl-CoA** or **carboxymethyl-CoA**, or **S- acetonyl-CoA** derivatives [96] or **D-serine** [5]. One should increase NADH, which is an endogenous citrate synthase inhibitor; malate, isocitrate or α ketoglutarate may support the formation of NADH. An inhibition of NADH deshydrogenase (complex 1) with **capsaicin,** or **rotenone**, or **amytal**, or **silymarin**, deserves some studies, it might also be useful to manipulate the CoQ quinone- semiquinone ratio. Slowing down aconitase with NO donors, might decrease the demand of acetyl-CoA for citrate condensation, but would favor the ATP citrate lyase route, which is a bad idea. **Target 8** concerns the accumulation of triglycerides in the tumor, an inhibition of ATP citrate lyase with **fluorocitrate** [97] and particularly with **hydroxycitrate,** seems very interesting to try. Aromatic ketones or chalcones also decrease triglycerides formed via the ATP citrate lyase route, **xanthohumol** could be tried [98]. Other compounds are **hesperidine methyl chalcone,** fish oil, and polyunsaturated fatty acids, **eicosapentaenoic** and **docosahexaenoic** that have triglyceride lowering properties. The effects of adiponectine [99] and the drug **honokiol** [100] (extracted from magnolia) seem to counteract alcoholic fatty liver and are particularly interesting to study in relation to SREBP/ PPARs and AMPdependend kinase controls over lipid metabolism. In the same line, it was observed that **indol-3-carbinol** a compound from cabbage had similar properties, inhibiting apolipoprotein B secretion [101]. The tripeptides (arg-gly-asp) called desintegrin obtained from snake venom [102] or a plant polyphenol **esculetin** [103] also decreases triglycerides. We have also seen that a phosphatase was involved in the synthesis of triglycerides; its inhibitors are **okadaic acid** and **calyculin**, or more selectively, **cantharidine** or **tautomycine** see ref [55]. The removal of triglycerides as VLDL by lipotropic factors, **choline, betaine**, **vitamin B12** is essential; compounds of bacterial origin such as **sespendole** also inhibit lipid droplets and would deserve a try see [104], **metformine** as well [105]. An action on this **target 9**, aims to attenuate the increased phosphorylcholine-choline kinase activity of tumors, it leads to lecithin (Kennedy pathway) and in parallel to triglyceride synthesis, and one would gain to inhibit choline kinase, with compounds such as **miltefosin** or **hexadecylphosphocholine** [106] or 5-aminoimidazole-4-carboxamide-1beta-d-ribofuranoside [107]. The activation of phospholipase C by alkylating platinum [108] suggests that cisplatine an anticancer drug may activate the phospholipase and decrease phosphatidylcholine. However, it should be taken into account that if the exogenous choline supply is deficient, membrane phospholipid break-down provides the necessary choline, this process is enhanced in tumors, one may then try to inhibit phospholipase D, with compounds such as **farnesol,** [109] or **polyisoprenyl phosphates** [110], or **N-acyl ethanolamine** [111]. The aim is to inhibit choline kinase and favor choline dehydrogenase and methylation pathways. **Tatget 10** deals with the methylation of PP2A that has to be helped, **choline, betaine, vitamin B12, folate, trimethylglycine**, would support this necessary methylation assembling the trimeric PP2A**; xylulose 5P** [112-113] or **ceramide** [114], or **N6 cyclo pentyl adenosine** [115] also activate the process reviewed in [15]. Methylated PP2A will then counteract the signaling kinases and activate of PK by dephosphorylating the PK dimers. It may also activate PDH and α ketoglutarate dehydrogenase, by dephosphorylating these enzymes as indicated for target 5. **Target 11**, deals with the reactivation of genes that are turned off (PETEN, P53, a "demethylase inhibitor gene" and many others), using HDAC inhibitors, to preserve the acetylation of histone tails, and help the action of transcription factors. The formation of ketone bodies (butyrate) an endogenous HDAC inhibitor should then be favored. **Butyrate or valproate esters** deserve a try, they would release after cleavage, the HDAC inhibi-

tor, butyrate or valproate, see [116]. The list of HDAC inhibitors is long: one finds **phenylbutyrate (amonaps);** or hydroxamic acid derivatives (**trichostatin**, **SAHA** or **vorinostat**, **scriptaid**); or epoxyketone-containing tetrape-
peptides (**trapoxine, chlamydoxin**); or cyclic tetrapeptides (**apicidin**, cyclic-hydroxamic- containing peptides, **CHAPS**); or **benzamides** and benzamide analogs MS-275. The silencing of a hypothetical "demethylase inhibitor gene" may favor the demethylation of adjacent genes that would become active, which is to avoid. In this respect, the properties of cytochrome P450 are interesting to consider, since it has in addition to the monoxygenase activity, a demethylase activity that one should then inhibit. **Target 12**: listed compounds decrease the demethylating action of cytochrome P450, **ketonazole**, **orphanedrine** [117] **quinidine**, **phenacetine**, **coumarine**, **quinine** or **methadone**, or those extracted from grapefruit **bergamottin** [118] they certainly deserve a try. The demethylation of adjacent genes and the recruitment of histone acetylase (HAT) could be a consequence of the HDAC mediated silencing of this demethylase inhibitor, which explains that in tumors, hyper and hypomethylated genomic regions coexist. The treatment with HDAC inhibitors, aims to cancel the silencing of genes like PETEN and others but the reactivation of the hypothetical demethylase inhibitory gene, may up-regulate the HAT recruitment over demethylated promoters and the activation of other genes, such as the hexokinase gene or the M2 PK gene, or genes controlling cell cycle proteins. We therefore suggest to reactivate silenced genes with HDAC inhibitors, and to inhibit in parallel Cytochrome P450 demethylase activity. Then, we may after some delay, continue the action on target 12, using HAT inhibitors. A host of compounds inhibits HAT, **anacardic acid** found in the cashew nut, produced by the cashew tree Anacardium occidentale [119] or found in seeds of Ginkgo biloba (120). We also have **garcinol** it comes from the fruit of the Mangoustan tree (Garcinia mangostana). Besides the effect on HAT, garcinol down-regulates the MAP kinase signals, and is a rich source of OH citrate that blocks the ATP citrate lyase, over expressed in tumors [121] Another interesting compound is a dietetary pigment **curcumin** from Curcuma longa; it is not only a HAT inhibitor but displays also protective effects against reactive oxygen species [122-123]. We have also extracts from Rosa rugosa Thumb, they are like the previous compounds HAT inhibitors but also anti-inflammatory [124]. To the list, we may add **Epigallocatechin-3-gallate** (green tea) it inhibits the acetylation of P65 and the activation of NFκB, it inhibits the stimulation of NFκB by TNFα and increases the cytoplasmic level of IκB [125]. To anacardic acid and garcinol, we may add a natural acylphloroglucinol, **myrtucommulone** extracted from myrtle Myrtus conmmunis L. [126]. Interesting derivatives of **isothiazolones** are also HAT inhibitors [127] we also mention here heparin like glycosaminoglycans that are more difficult to use [128] and **4-hydroxyquinoline** [129]. The Insulin tyrosine kinase receptor signals is the next **target 13**, many compounds have been developed: **glivec** and others (**sorafenib**, **gefitinib**) they inhibit the tyrosine kinase step, inhibitors of the PI3 kinase branch are also covered by target 13, known inhibitors are **wortmannin**, **LY294002**. There too, the tyrosine kinase signal stimulates phopholipase Çγ; inhibitors such as **eupatilin** [130] or **uncarinic acid** [131] deserve a try. Interesting anticancer drugs such as **genistein** and **esculetin** that inhibit cell growth [132] relate to coumarine (vit.K), flavonoides and benzopyrone. The antimalaria drugs **quinine** and **quinacrine** also inhibit phospholipase A2 that is activated in tumors, generating inositol-3P (IP3) and diacyl glycerol (DAG) that have to be decreased, since they support the effects of tyrosine kinase signals and the activation of PKC. The next section deals with **targets 14, 15,** and **16.**

Growth hormone physiology: other pharmacological targets affecting tumors

In the brain, dopamine-releasing neurons stimulate hypothalamic neurons that release growth hormone releasing hormone GHRH. Dopamine activates hypothalamic D2 receptors coupled to Gi proteins. Thus, the decrease of adenylate cyclase activity and cAMP in hypothalamic neurons closes K+ channels; a depolarization opens Ca++ channels, which eticits GHRH release. In order to decrease GHRH release, one may impair the re-uptake of dopamine with **mazindole**, or inhibit the D2 receptors with **sulpiride**. On the contrary, at the hypothalamic level D2 agonists (**bromocriptine**) increase GHRH release, eliciting the release of GH. The hormone GHRH acts upon pituitary receptors coupled to Gs proteins. Thus, an increase of cAMP stimulates protein kinase A (PKA) and enhances in pituitary cells, the synthesis and release of GH. However, the pituitary cells are also equipped with D2 receptors coupled to Gi, proteins, which decreases cAMP, counteracting the action of GHRH. The overall effect of bromocriptine is a decrease of GH in spite of the increased GHRH it elicits, explaining the utilization of bromocriptine for treating Acromegalia, see figure 5.We have studied above the different effects of GH. In the liver, GH activates membrane receptors that dimerize, to trigger STAT kinase signals and the transcription of IGF. We have also discussed the possible induction of IGFBP in dividing cells by butyrate coming from the liver, and proposed a mechanism for asymmetrical mitosis. Well **pegvisoman** is an interesting drug that blocks the induction of IGF by GH. It may be advantageous to facilitate in parallel the production of IGFBP with **butyrate** or other HDAC inhibitors (**trichostatin SAHA**, **valproate**, **benzamide** etc...). Avoid arginine, since arginine increases

GH release. Moreover, a diet poor in arginine affects particularly tumor cells (see addendum). An up – regulation of IGFBP with **apigenin**, a falavonoid present in fruits and vegetables, probably explains the anticancer properties of the compound [133]. There are many other up-regulators for IGFBP: **bicalutamide-(casodex**) [134]; we find also **omeprazole** [135], **traconol** from grape seed [136] and **silibinin** [137]. It would be interesting to study, cAMP increasing drugs (8- Br- cAMP) [138] Nitric oxide (NO) inhibition [139] (aminoguanidine), or the effect of RRR- alpha- vitamin E [140] and vitamin D analogue EB1089 [141]. Note that hypoxia, which induces the fetal gene cassette, might increase in parallel the IGFBP protector. In addition, it seems that adjuvants induce IGFBP. It would be particularly interesting to decrease GH with **somatostatin** a hormone secreted by neurons of the periventricular nucleus in the hypothalamus. Neurosecretory endings release somatostatin in the blood vessels of the hypothalamo-portal system, which inhibits the release of GH from the somatotroph cells. The pancreas δcells but also the stomach and intestine release Somatostatin. Inhibitors of the pituitary gland are here essential; **octreotide** is an analog of somatostatin. One may also test niacine already mentioned, and **POP** (**paraoxy propiophenone**) this drug inhibits GH release; it was studied in parallel to thyroxin on tadpole metamorphosis, leading to frog dwarfs. Remember also that fatty acids retroinhibit GH release. Other compounds **perillylic alcohol** (lavender) or extracts from orange skin or cabbage may act like POP on GH release. Other drugs such as psoralens (**bergapten**) used for treating psoriasis decrease keratinocyte cycling, and IGF receptors, they may then attenuate an exagerated IGF effect [142]. It is also possible to try plasmine serine-threonine proteases inhibitors, since this protease hydrolyses IGFBP. To be studied also, the effect of kinases and phosphatases on the activity of IGFBP. All these compounds cover an action on targets **14, 15,** and **16** of figure 6.

We know that hypoxia activates HIF1-Von-Hippel factors increasing the transcription of VEGF, glycolytic enzymes, NO Synthase, inflammation components (the fetal gene cassette) as discussed above. These conditions adapt cells to hypoxia favoring a glycolytic fermentation, while increasing the blood supply (dilatation an angiogenesis take place). Hypoxia plays a prominent role in cancer, in retinopathy, in juvenile fetal cell metabolism. Here, the growth factor involved is VEGF rather than GH- IGF. Prolonged hypoxia leads to digital clubbing (finger hippocratism) [143] it is often the sign of a severe disease.

The mitotic action of IGF depends of IGFBP, whereas VEGF induced mitotis escapes from such controls, favoring perhaps a symmetrical mitosis, in which both daughter cells become stem cells. It seems then adequate to use also anti-VEGF, anti-angiogenic drugs, since hypoxia favors VEGF actions. Note that inhibitors of GHRH release seem to decrease VEGF [144]. In conclusion, it is essential to preserve the asymmetry of mitosis and important to spare the capital of stem cells, avoiding for example GH injections, for the sake of feeling better, this consumes the stem cell capital and decreases longevity. Many works indicate that a decrease of IGF increases longevity via the inactivation of the klotho gene for example [145]. Moreover, an increase of IGFBP (presumably via the action of ketone bodies) may block IGF and thus lead to an increased longevity. This establishes a link with studies on the effect of fasting and hypo caloric diets on longevity since fasting increases ketone bodies.

Cancer prevention, immune destruction of tumor cells: more drugs

The procedure proposed by Gernez for preventing cancer did not receive the required attention. The procedure done once a year, consists of three essential parts : **first** a low calorie regime applied during a fasting period, "this is recommended by most religions", **second** one should eat more fruits, vegetables, less meat, consume products rich in magnesium, vitamins C, E, selenium (Brazil nuts are rich in selenium). The **third** point is essential, but more difficult to apply without adequate studies on animal models; it aims to kill once a year eventual tumor cells, by giving for a few days an anti-mitotic, colchicine for example. Gernez mentions that chloral, anciently used for calming mental patients, also protected them from cancer, because it has anti-mitotic properties. The idea being to kill the very first cancer cells that inevitably appear, and if missed the first year, one would catch them the next year etc… As long as the tumor cell colony remains below 100000 cells, it is possible to eliminate it. Imagery cannot detect such a small colony, it detects masses of $1cm^3$ (ie.10^9cells) at a stage where reversibility is more difficult.

How can we explain the possible preventive action of this procedure, in relation to metabolic features of tumor cells? The first point is evident, fasting increases ketone bodies such as butyrate, which is a histone deacetylase (HDAC) inhibitor, this keeps histone tails acetylated, loosening the DNA thread, rendering it accessible to transcription factors, which cancels the silencing of several genes starting the tumor process. The second part of the procedure takes advantage of the anti- inflammatory properties of flavonoids found in fruits and vegetables. Flavonoids would cancel the effects of hypoxia on the expression of a set of genes: cyclooxygenase (COX), VEGF, NOsynthase, glycolytic enzymes, induction of fetal genes. We have seen that fetal M2 PK expression is a typical feature of tumor cells. These groups of genes adapt tissues to hypoxia, since they support vasodilata-

tion, angiogenesis, inflammation, bringing more blood to the hypoxic tissues, while glycolytic energy compensates for the decreased oxygen availability and oxidative metabolism. In some way, the hypoxic tissue returns to a situation prevailing in the fœtus, "an aquatic creature that draws oxygen from his mother's blood". The anti–cancer properties of 'non-steroidal anti-inflammatory drugs,'' NSAIDS, results from the inhibition of the COX gene included in the"hypoxic fetal gene cassette" they decrease pro-inflammatory prostanoids. Moreover, magnesium may inhibit calcium dependent phospholipases forming arachidonic acid, the prostanoid precursor. The protective action comes also from the quenching of superoxides and peroxynitrite, ensured by vitamines C, E, and selenium, which counteract the effects of hypoxia on a poor reduce ation of oxygen giving superoxides. One may strengthen this second line of protection with methyl donors, vitamin B_{12}. They would attenuate, via methylated PP2A, the signaling kinases, activated by insulin or IGF, and regulate the supply of glucose, anabolism and mitosis. The third point of the procedure is more difficult to apply and deserves additional experiments. The hypothesis is that tumor cells appear frequently but we constantly eliminate them. If the physiological process that normally eliminates such cells fails, one may still use an anti-mitotic for eliminating them before they reach a critical mass. A mild and short anti-mitotic treatment aims to kill once a year, the very first cells that escape the immune surveillance. If missed the first year, one may catch them the next year etc… this aims to block the formation of a tumor that

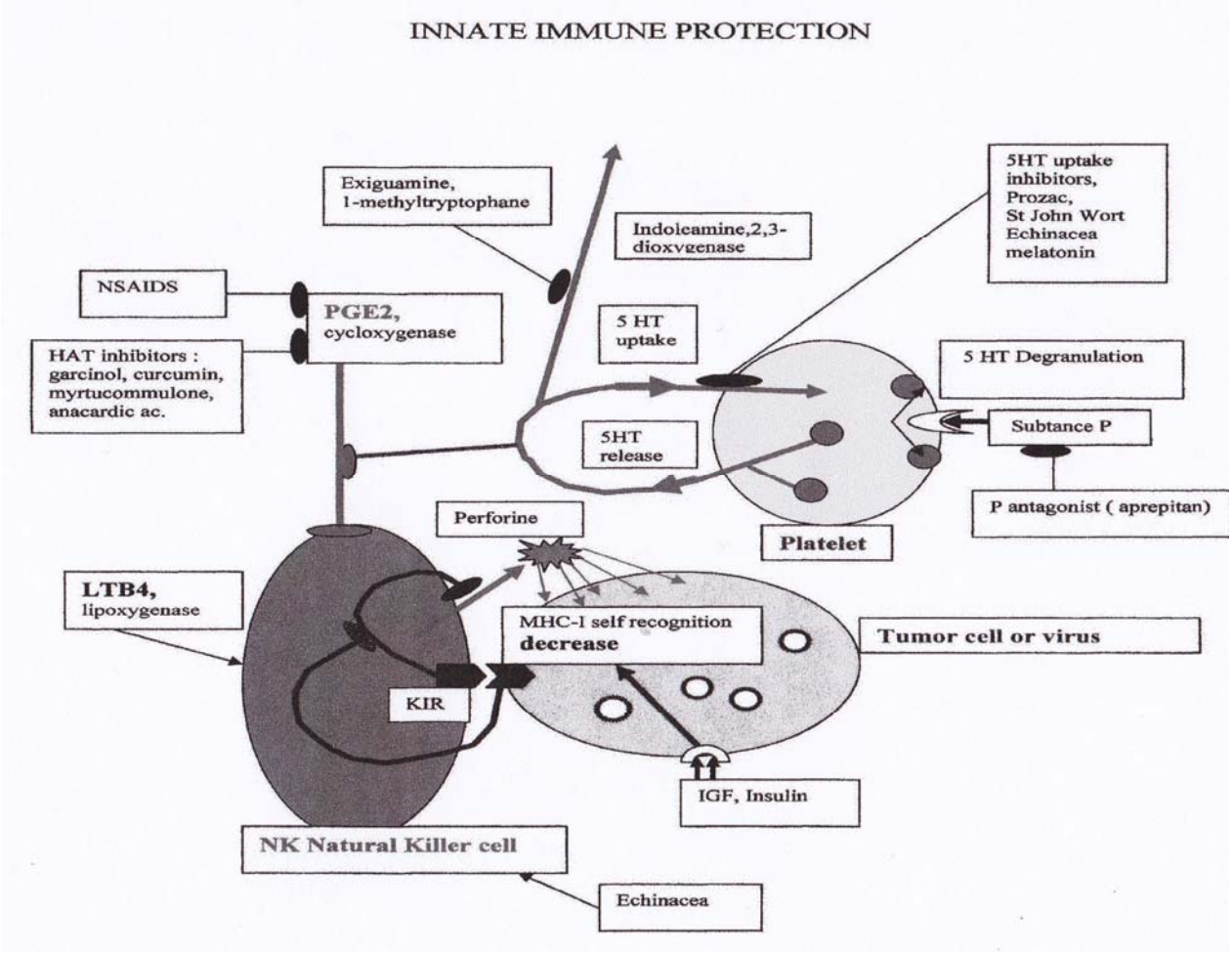

Figure 7 Innate Immunity.

Tumor cells or virus-invaded cells would display lower MHC-1 levels, the major histocompatibility complex, which is a self-recognition device. The consequence of the MHC-1 decrease is to trigger the "Natural killer" (NK) immune protection, because MHC-1 stops to neutralizing KIR receptors on NK cells, which elicits the release of perforines by NK cells, that destroy cells poor in MHC-1.

The NK protection is activated by leucotriene LTB4 formed by lipoxygenase, and inhibited by the fever prostaglandin PGE2 formed by cycloxygenase. Hence, inhibitors of PGE2 and cycloxygenase trigger the NK protection; this is the effect of non-steroid anti-inflammatory drugs (NSAIDS) aspirin, ibuprofen or of some histone acetylase (HAT) inhibitors (curcumin, garci-

nol etc...). It is also particularly interesting to increase serotonin (5HT) that neutralizes the inhibition of NK cells by PGE2. Echinacea extracts, 5HT uptake inhibitors (prozac, melatonine) boost the release of 5HT from platelets, and particularly if platelets are full of 5HT before reaching the site, (a substance P antagonist, aprepitan, avoids their premature degranulation). Finally 5HT can be preserved from its enzymatic conversion into quinolinic acid by inhibiting indoleamine,2,3-dioxygenase with exigyamine or 1-methytryptophane. Echinacea; melatonin; 5HT uptake inhibitors;, substance P antagonists; and HAT inhibitors, boost the immune surveillance.

would be difficult to eliminate. This third point of the procedure is certainly an interesting possibility that may back-up the immune surveillace when it fails. It deserves a try on animal models.

It is here useful to discuss the immune surveillance that should eliminate tumor cells and understand why it sometimes fails (see figure 7). It is probably frequent, that cells overcome the regulations described, adopting a perverted metabolism typical of tumors. Normally, the immune system recognizes such cells by their surface tags and eliminates them. What is the mechanism that fails if such cells survive? In the tumorigenic mechanism described, we have given to insulin signals attenuated by methylated PP2A, and to Growth hormone-IGF signals controlled by IGFBP capping, a prominent role. Well, insulin and IGF down regulate in parallel to their metabolic action, the expression of MHC-1, the major histocompatibility complex class1, our self-recognition device [146]. It is like if an excess IGF, or insulin signals, which starts the tumor cell deviation, also tells that such cells are no longer our self. In this case, the innate immunity protection should operate, and the so-called Natural killer cells (NK) should destroy such cells with low MHC-1 [147], but for some reason, they do not do their job, when cancer takes over, (see http://wikipedia.org/wiki/Natural_killer_cell). We know that NK cells are stimulated by leukotriene LTB4 generated by lipoxygenase [148] but are inhibited by PGE2 the fever prostaglandin formed by cycloxygenase [149]. It is thus probable that the failure of NK cells comes from their inhibition by PGE2. Inflammation seems to control the process switching from innate to specific immunity via the LTB4/ PGE2 ratio. As long as LTB4 is above PGE2, (lipoxygenase greater than cycloxygenase) NK cells and innate immunity dominate. However, if PGE2 increases, it switches off the innate mechanism, opening the way to specific immunity involving cytotoxic and helper T cells, B cells, polynuclear leucocytes, myeloperoxidase.

The consequence of a decreased NK activity is that eventual tumor cells, may escape apoptosis triggered by NK cells. The first line of protection is defeated. Thus when metabolic signals (IGF or insulin for example) are not regulated, they become oncogenic, starting the tumor mechanism; fortunately, these signals decrease in parallel MHC-1, which induces the NK protective mechanism, except when inflammation triggers via an excess of PGE2, an inhibition of NK cells, allowing tumor cells to develop. It is then crucial to inhibit the increase of PGE2, in order to keep active the NK protection, which eliminates the tumor cells that are poor in MHC-1. The NK cells attack the tumor cells by secreting granenzymes, perforine, proteases that elicit the apoptotic mechanism in tumor cells. Indeed, when MHC-1 decreases, receptors on NK cells, named KIR receptors, are no longer inhibited, allowing the release of perforine, this triggers the apoptose of cells poor in MHC-1. The innate immune protection by NK cells operates also for virus-infected cells and limits the propagation of viruses by triggering apoptosis in infected cells, including T lymphocytes invaded by the HIV virus.

We have given to the NK innate mechanism, a prominent role for eliminating tumor cells; it would be interesting to find drugs that boost this mechanism? We did mention the protection by NSAIDS they inhibit the cycloxygenase (COX) and thus preserve the NK function, because they cancel the inhibition of PGE2 over NK cells. However, NSAIDS, **aspirin**, **ibuprofen**, are not easy to use because of side effects. Serotonin uptake inhibitors (**prozac**), St John's Wort tea containing **hypericine**, **adhyperforin**, **hyperforin** are antidepressors that increase extracellular serotonin, [150]. They also support the production of NK cells, by inhibiting their PGE2 inhibitor [151]. **Melatonin** (found in walnuts) [152,153] and perhaps another indol, **indol 3 carbinol** (found in cabbage) may similarly help the formation of NK cells, probably via an inhibition of serotonin uptake. A substance P antagonist (**aprepitan**) also increases NK function. This is unexpected since substance P antagonists normally decrease serotonin release from platelets. The explanation is that it is necessary to bring on the site, platelets that have not been degranulated and depleted by substace P. The antagonist of substance P keeps them full, allowing platelets to release locally, greater serotonin amounts in presence of serotonin uptake inhibitors. A local and sustained increase of extra-cellular serotonin inhibits PGE2 and activates NK cells. In this respect, a substace P antagonist such as aprepitant could be associated to a serotonin uptake inhibitor, **fluoxetine (prozac)**, **paroxetine** and similar compounds. Other inhibitors of PGE2 are anti-fever compounds such as **cryogenine**, an alkaloid of Hemia Salicifolia, known as Sinicuichi of Aztecs, and Mexican shamans. Auguste Lumière developed the drug; he did many interesting pharmacological contributions, in parallel to the invention of cinematography with his brother Louis. A particularly interesting procedure for enhancing

the innate NK protection is a miracle herb, **Echinacea.** It decreases PGE2, by its alkamides, enhancing NK cell function. It also contains arabinogalactane, which stimulates the formation of NK cells, via a cocktail of lymphokines (Interferon β γ, TNFα, IL1), [149]. In association with **melatonin**, or **cryogenine**, the effect of Echinacea on NK cell mediated protection might be useful. It is remarkable that the HAT inhibitors that we listed above: **anacardic** casew extract **garcinol** [154-156] from the Mangoustan tree, display similar anti-inflammatory properties. The compounds might inhibit the expression and synthesis of PGE2, the fever prostaglandin formed via cycloxygenase. The protective action of **curcumin** in prostate cancer is in this respect particularly interesting [155] since it should enhance the NK mediated destruction of tumor cells or viral infected cells. **Acylphloroglu**

cinol (**myrtucommulone**) extracted from myrtle Myrtus conmmunis L. has similar actions [154-156]. Genes supporting the expression of inflammatory proteins are part of this "fetal genes cassette", activated by hypoxia, and indeed, their epigenetic reprogramming depends of HAT mediated histone acetylation, explaning why HAT inhibitors antagonize inflammation by turning off the inflammation genes. Another compound, **licofelone** 2-[6-(4-chlorophenyl)-2, 2-dimethyl-7phenyl-2, 3-dihydro-1H-pyrrolizin-5yl] acetic acid suppresses PGE2 synthesis [157].

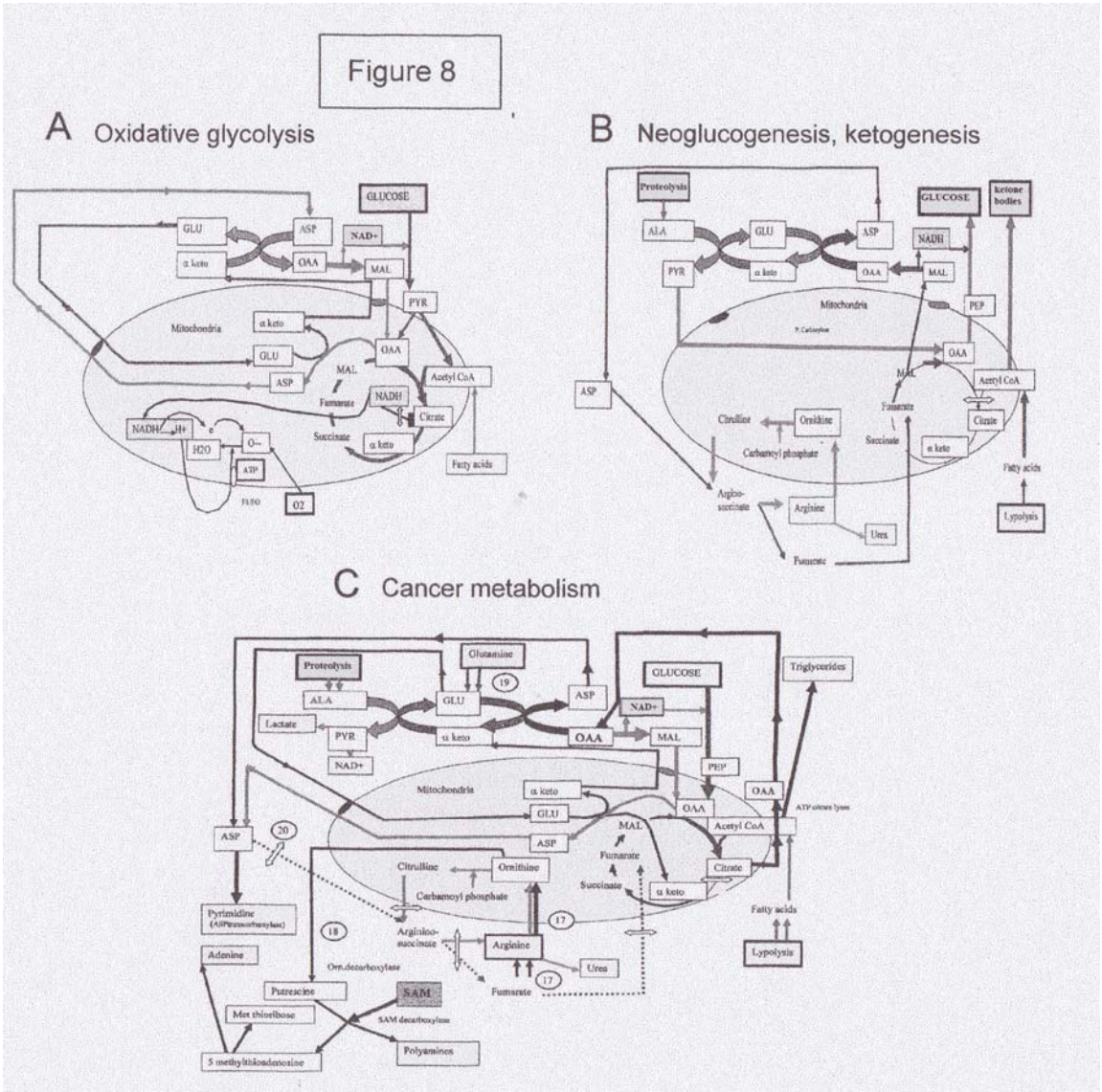

Figure 8 Glycolysis- Neoglucogenesis-Tumor metabolism

A: Oxidative glycolysis and malate-aspartate shuttle function*. Glycolysis requires NAD$^+$ at the glyceraldehyde dehydrogenase (Gl dehydrogenase) step; the malate/aspartate shuttle drives the formation of cytosolic NAD+. Substrates go through*

the shuttle via two mitochondrial membrane antiporters, one exchanges malate (MAL) and α ketoglutarate (α keto), the other exchanges glutamate (GLU) and aspartate (ASP). Malate dehydrogenase works in opposite directions inside and outside the mitochondria. Outside, it converts oxaloacetate into malate, forming NAD^+ in the cytosol; inside it converts MAL into (OAA) and NADH. In the cytosol, NAD+ pushes glyceraldehyde dehydrogenase in the glycolytic direction. When glycolysis operates, pyruvate kinase (PK) the last enzyme of glycolysis, converts irreversibly phosphoenolpyruvate into pyruvate. The latter, feed the Krebs- Henseleit cycle with acetyl-CoA via pyruvate dehydrogenase (PDH). Acetyl- CoA also comes from fatty acids β oxidation. The condensation of acetyl- CoA and OAA into citrate starts the cycle. There are several OAA sources; OAA may result from the transamination of aspartate and α ketoglutarate. Other OAA sources being Pyruvatecarboxylase (it operates essentially in neoglucogenesis) or PEP carboxykinase. The shuttle was "invented" because the mitochondria are not permeable to NAD^+ or NADH co-factors, or to oxaloacetate.

B: Neoglucogenesis -Transaminases fluxes*.The process mobilizes body stores for making nutriments, glucose and ketone bodies. For making glucose from pyruvate, a barrier to overcome is pyruvate kinase, which is not reversible, and cannot convert pyruvate into phosphoenolpyruvate. Two other enzymes do the work. First pyruvate carboxylase (P carb) only found in mitochondria, it receives pyruvate, derived from alanine, via proteolysis (in starvation) second phosphoenolpyruvate carboxykinase (carb kin, often abbreviated PEPCK), which is both cytosolic and mitochondrial. Pyruvate carboxylase drives the flux, converting pyruvate into oxaloacetate (with the help of biotin) then phosphoenolpyruvate carboxykinase follows, converting oxaloacetate to phosphoenolpyruvate (this enzyme needs GTP). The phosphoenolpyruvate leaves the mitochondria and gives after several steps glucose. As for malate, it quits the mitochondria and forms oxaloacetate in the cytosol (via malate dehydrogenase) and NADH required by gl.dehydrogenase in the neoglucogenic direction. On the gluconeogenic route, two other barriers will have to be overcome by phosphatases, at the fructose 1, 6 bis phosphate and glucose phosphate steps (not shown). The pathway would not operate if pyruvate kinase were not at rest, since phosphoenolpyruvate would give back pyruvate. The pyruvate kinase blockade is precisely what does cAMP when it signals, in the liver, that it is necessary to synthesize glucose, after starvation for example. The pyruvate entering the mitochondria gives OAA via pyruvate carboxylase. Pyruvate dehydrogenase (PDH) is at rest in a phosphorylated form. Citrate condensation is off. Hence, acetyl-CoA will form ketone bodies that are like glucose, nutriments for most cells.*

The transaminases, ALA tansaminase and GLU/OAA transaminase operate in a direction consuming the OAA formed by MAL dehydrogenase, which feeds ASP in the urea cycle. The urea cycle is indeed functional in neoglucogenesis. The argininosynthase step follows, and then argininosuccinate splits, giving a fumarate input to the Krebs- Henseleit cycle, while arginase splits arginine into urea and ornthine. In the mitochondria, the carbamoylation of ornithine into citrulline closes the urea cycle.

C: Tumor cell metabolism *Figure 1 represents the PK bottleneck; we complete the metabolic picture by indicating that in tumors the malate-aspartate shuttle operates with an aberrant transaminase orientation depleting body stores.*

Glycolysis requires NAD+, it comes from Pyruvate to lactate conversion (Warburg effect) and from malate dehydrogenase working in the cytosol from the OAA to MAL direction. Then MAL moves in the mitochondria through the shuttle giving back OAA. In tumors, there is an active citrate condensation between OAA and acetyl-CoA provided by fatty acids and lipolysis. Other OAA sources are via PEP carboxy kinase or via the malic enyme. Citrate quits the mitochondria and forms in the cytosol, via ATP citrate lyase; acetyl-CoA and OAA (target 8 of figure 6). The acetyl- CoA will make fatty acids and triglycerides. Above all, the other product of ATP citrate lyase OAA seems to have an essential role, since the OAA pressure pushes the transaminase chain in a direction usually associated to gluconeogenesis! This consumes protein body stores, proteolysis gives ALA it is essential for tumors, like glutamine. The output of the transaminase chain is ASP the flux joins with ASP coming from the shuttle and feeds the demand of ASP transcarbamylase, starting the synthesis of pyrimidine bases. In tumors, ASP in not used by argininosuccinate synthetase, which is blocked, interrupting the urea cycle. Arginine gives ornithine via arginase. Ornithine is decarboxylated into putrescine by ornithine decarboxylase, polyamines spermine spermidine form, via SAM decarboxylase. Arginine deprivation would affect this step. The other product of the polyamine pathway is 5 methylthioadenosine, it feeds adenine in the purine pool. The consummation of bases by tumors for DNA and RNA synthesis is elevated. The destruction of SAM via the polyamine pathway impairs methylation processes as discussed above, particularly for PP2A, which removes the brake over signaling kinases.

The figure 8C indicates possible therapeutic targets numbered ***17, 18, 19, 20,*** *following the numbers of targets of figure 6.*

In several neurological diseases (depression, or AIDS dementia) the activity of an enzyme: indoleamine, 2, 3-dioxygenase, increases. This enzyme converts tryptophan to N-formylkinurenine. The latter, then gives picolinic or quinolinic acid, which gets elevated in body fluids. The resulting decrease of serotonine attenuates the NK protection mechanism against viral infections or tumor cells. It would then be useful to try inhibitors of this enzyme for boosting the NK protection, **exiguamine A**, extracted from the marine sponge Neopetrosia exigua, [158] **or 1-methyl tryptophane** [159] may be added to the list. It is possible that **indol 3carbinol** found in cabbage inhibits this enzyme. It is worth testing some other protective asteraces as well, among them, the Greater burdock or Centaurea imperialis [160] they contain an interesting polyphenolic lignan (**arctiin**), http:// en. wikipedia.org/ wiki/Arctiin. Other interesting herbal medications include Latrix occidentalis, Crinum asiaticum; they are in the

traditional medicine, see [161]. A flavene derived fom genisteine (**phenoxodiol**) [162] also enhances the NK function and displays anti-cancer properties. One should study as well the anti-inflammatory properties of Borage and fish oil. It may be interesting to evaluate MHC-1 antigen presentation and expression, [163] to compare saturated palmitate to oleate [164]. A decrease of the cell surface concentration of MHC1 in tumor cells would boost the NK protection; it may be interesting to find the effects of tunicamycine, brefeldin and drugs controlling the protein traffic. An anti-thyroide (methimazole) seems to decrease MHC1.

http://www.patentstorm.us/patents/5556754.html,

In sum, the innate immunity mediated by NK cells, kills tumor cells that might form more frequently than thought. If a weakening of the NK protection following an inflammation takes place, tumor cells escape and develop.

Addendum: the malate/aspartate shuttle and polyamines in cancer

Several aspects of tumor cell metabolism, transamination and the malate/aspartate shuttle need an additional description indicating other potential therapeutic targets. We first need to recall the shuttle function in normal cells, in the course of oxidative glycolysis. Then, we shall switch to a neoglucogenic and ketogenic metabolism associated to an up-regulation of the urea cycle. The portions of such pathways that are functional, or at rest, in tumor cells show the link between the mitochondria citrate efflux, glutaminolysis, polyamines and the Warburg effect. The mitochondria internal membrane is not permeable to NADH or NAD^+ co-factors, they will have to be oxidized or reduced, in situ on both sides of the mitochondria membrane, by specific substrates that go through "shuttles", across the mitochondria membrane. The enzymatic oxido-reduction of the transported substrates regenerates on each side of the membrane, the co-factors. The work of the malate-aspartate mitochondria shuttle is illustrated in figure 8 A, NAD^+ appears in the cytosol, allowing glycolysis to proceed (at the glyceraldehyde dehydrogenase step) while NADH, builds up in the mitochondria, forming a potential energy source. The NAD^+ / NADH ratio regulates glycolysis and its continuation by oxidative metabolism. Two mitochondria membrane antiporters support the shuttle function, one exchanges malate (MAL) for α ketoglutarate (α keto), the other exchanges glutamate (GLU) for aspartate (ASP). Malate dehydrogenase present on both sides of the mitochondrial membrane drives the flux through antiporters. This enzyme, converts oxaloacetate into malate, it forms NAD+ in the cytosol, and does the reverse inside the mitochondria forming NADH. The other enzyme pulling the flux of substrates through the antiporters is a transaminase. When glycolysis operates, it transaminates aspartate and α ketoglutarate, to form glutamate and oxaloacetate in the cytosol, and does the reverse in the mitochondria. Hence, if glycolysis is on, NAD^+ forms in the cytosol, if glycolysis switches to gluconeogenesis; NADH is preserved and utilized in the cytosol. These two co-factors control the direction of glyceraldehyde 3-phosphate dehydrogenase, (Gl dehydrogenase), which converts glyceraldehyde 3-phosphate (Gly 3P) into 1-3 diphosphoglycerate (1-3DPG) in the glycolytic direction, using NAD^+, and does the opposite, utilizing NADH, for gluconeogenesis. The intra- mitochondria NADH potential controls the citrate condensation and entry in the Krebs – Henseleit Cycle. When ATP synthesis takes place, the inward flow of protons through the F1/Fo ATPase generates ATP within the mitochondria, while electrons reach oxygen via the succession of complexes belonging to the electron transport chain. The energy of the NADH potential is in fine recovered as ATP, and water (figure 8A). In Hypoxia, NADH probably builds up, closing the citrate condensation of the Krebs cycle, and oxidative metabolism. Lactate fermentation starts, generating the required NAD+. The glycolytic source of ATP takes over, substrate phosphorylations occuring at Phosphoglycerate kinase and Pyruvate kinase steps of the glycolytic, lactate fermentation process.

Switching from glycolysis to neoglucogenesis is complicated since pyruvate kinase is not a reversible enzyme (figure 8B). How will the cell overcome this first obstacle to go from pyruvate to phosphoenolpyruvate? Recall that this is achieved by two enzymes, one is pyruvate carboxylase (P carb) an enzyme requiring ATP, biotin-CO2 and only present in the mitochondria, where it forms oxaloacetate, the other is the phosphoenolpyruvate carboxykinase, which is present both in the mitochondria and in the cytosol; it converts the oxaloacetate into phosphoenolpyruvate and requires GTP. Hence, in glucose synthesis, pyruvate enters the mitochondria, where it forms oxaloacetate, via pyruvate carboxylase. The oxaloacetate then gives phosphoenolpyruvate via phosphoenolpyruvate carboxykinase and quits the mitochondria, to follow the glucose synthesis pathway. Alternatively, oxaloacetate may give malate via malate dehydrogenase, and quits the mitochondria, to reform OAA in the cytosol, then PEP to join the gluconeogenic route. This system copes with the fact that mitochondria are not permeable to oxaloacetate.

Gluconeogenesis would be inefficient if pyruvate kinase reconverted the phosphoenolpyruvate formed into pyruvate, it is then necessary to block the enzyme. This is precisely what does cAMP activating gluconeogenesis in the liver. NADH and ATP, may also inhibit liver pyruvate kinase. During starvation, proteolysis of muscle proteins stores give amino acids, alanine is transaminated into pyruvate feeding the pyruvate carboxylase reaction. Alanine transamination drives the flux through the

transaminases as indicated in the figure 8B. The efflux of ASP feeds in the urea cycle that is very activate in gluconeogenesis. The figure indicates the citrulline aspartate reaction forming argininosuccinate, in the cytosol, and its hydrolysis into arginine and fumarate. The latter enters in the mitochondria and the Krebs-Henseleit cycle, forming Malate and OAA. As for arginine, it gives via arginase, ornithine and urea. Ornithine is carbamylated into citrulline in the mitochondria closing the urea cycle. Because gluconeogenesis consumes OAA, the citrate condensation starting the Krebs cycle becomes slow, acetyl-CoA coming from the mobilization of lipid stores and β oxidation of fatty acid accumulates, forming ketone bodies, essentially in the liver, they serve as nutriments for most cells.

We recall this here because tumors utilize in a special way parts of the pathways described in figures 8A, B. We have seen how tumors overcome the PK and PDH bottlenecks and evidently, the increased glucose influx, favors the supply of substrates to the pentose shunt, as pentoses are indeed necessary for supporting the synthesis ribonucleotides DNA and RNA in tumors. The figure 8 C schematizes what we draw from several observations. First, recall that citrate condensation in increased in tumors; and that citrate quits the mitochondria to give via ATP citrate lyase, acetyl-CoA and OAA; we have described above these observations. We have seen that acetyl-CoA supported the synthesis of fatty acids and the formation of triglycerides. However, the other product of the ATP citrate lyase reaction: OAA, increases as well, driving the transaminase cascade (ALAT and GOT transaminases) in a direction that consumes GLU and glutamine and converts in fine ALA into pyruvate and lactate plus NAD+ see figure 8 C. Another source of NAD+ comes from Mal dehydrogenase, which converts part of OAA into malate; the latter, enters the mitochondria via the shuttle and gives back OAA to feed the citrate condensation. I may be interesting to inhibit cytosolic malate dehydrogenase with **D malate**, **humic acid**, (thyroxine derivatives and others to test), this may in fine, strengthen the necessary inhibition of ATP citrate lyase. Glutamine will also provide amino groups for the de novo synthesis of purine bases particularly active in tumors. The figure 8C indicates that ASP shuttled out of the mitochondrial, joins the ASP formed by cytosolic transaminases, to feed the synthesis of pyrimidine bases via ASP transcarbamylase, a process also enhanced in tumor cells. This is associated in tumors, to a silencing of the argininosuccinate synthetase step of the urea cycle [165-167]. This blockade also limits the supply of fumarate to the Krebs cycle. The latter, utilizes the α ketoglutarate provided by the transaminase reaction, since the source of α ketoglutarate via aconitase is probably at rest. Indeed, NO and peroxynitrite increase in tumors and probably block aconitase. An essential reaction is the cleavage of arginine into urea and ornithine. In tumors, the ornithine production increases, following the polyamine pathway. Ornithine is decarboxylated into putrescine by ornithinedecarboxylase, then it captures the backbone of SAM to form polyamines spermine then spermidine, the enzyme controlling the process is SAM decarboxylase. The other reaction product, 5-methylthioadenosine is then decomposed into methylthioribose, and adenine providing purine bases to the tumor. Remember here that we have given to SAM a major role in the methylation of PP2A, the destruction of SAM (allowing the recovery of adenine) may then aggravate the situation, removing the PP2A brake over mitogenic MAP kinases, favoring like polyamines, the mitotic carcinogenic process. The pathways represented figure 8 C indicate that tumors will be sensitive to several treatments affecting these new targets, that are numbered as **targets 17, 18, 19, 20** in figure 8 C. First: the polyamine pathway renders tumor cells depend of arginine-ornithine, this is not the case of normal cells, an **arginine deprivation** diet, acts on **target 17** of figure 8 C, and this may selectively affect tumor cells, and seems particularly interesting, see for example [165-167]. A more efficient arginine deprivation is possible, using arginine depleting enzymes as **pegylated argininedeiminase** (polyethyleneglycol-associated preparations) that are presently evaluated [168-170]. Second: it should be useful to inhibit the production of ornithine. In tumors, ornithine comes essentially via arginase rarther than from transamidinase [171]. Thus, arginase inhibitors such as **norvaline** [172] or **nor-NOHA** (N-omega-hydroxy-nor-l-arginine) or **boroarginine** deserve a try. [173]. This action is also partly dealt in target 17 of figure 8 C. Third: the polyamine pathway (**target 18**) is sensitive to ornithinedecarboxylase inhibitors, such as **DFMO** (2-difluoromethylornithine) that are under evaluation, in association with other agents. Inhibitors SAMdecarboxylase are useful to try since they would stop the destruction of SAM, they are, **MGBG** methylglyoxal bis (guanylhydrazone) and 4-amidinoindan-1one-2'-amidinhydrazone [172]. Histidine decarboxylase inhibitors (**alfa-fluoromethylhistidine**) act on the same step (175). The protective anti-cancer action of green tea**, resveratol** or **geraniol**, might result from the decrease of ornithine [176]. Fourth: the starters of the process depends as indicated figure 8 C of the OAA supplied by ATP citrate lyase that has to by inhibited with **OH citrate** for example. It is crucial to block the transaminase chain at the alanine transaminase step **with aminooxacetic acid** and other compounds, as discussed above. However, it is also clear that the glutamine input should be limited (**target 19** figure 8 C) using glutaminase inhibitors, such as **DON**: 6-diazo-5-oxo-l-norleucine, which decreases proliferation and induces a senescent cell phenotype [177]. Antiglutamate drugs, **riluzole** and **naftazone** would also act on this step. Five: an up-regulation of argininosuccinate synthetase with **troglitazone** was observed [178] this would attenuate the polyamine route but

other less hepatotoxic derivatives should be tried (**target 20** figure 8 C).
In fact, the pathways found in fig 8 represent a system able to generate energy (when the Krebs cycle turns) or nutriments (depletion of body stores and interrupted citrate condensation). The system may also regenerate stores and form new substance, in this case, citrate condensation takes place, but isocitrate formation and aconitase are blocked; citrate quits the mitochondria feeding ATP citrate lyase and the lipogenic route etc... NADH, ATP/ AMP ratio and NO control the switches that orient the system toward the production of energy, nutriments; or alternatively, body stores and substance. In cancer, switches are set in a position that consumes body stores for making the tumor substance.

Conclusions

A. Multifactorial therapy

As a complement to radiotherapies or chemotherapies, we propose to try, after validating it on animal models [179] a sequential metabolic therapy aiming to reestablish a normal cell metabolism and then eliminate those cells that do not recover.

First part: metabolic correction

One would give compounds counteracting the consequences of the glycolytic bottleneck, one must activate Pyruvate kinase and Pyruvate dehydrogenase with **polyethylene glycol** and **lipoic acid** respectively or with the compounds listed in the text, the best combination should be tested and found. Glycolysis should be attenuated (**manoheptulose, lodinamine** and may others). The decrease of NAD+ by LDH inhibitors (**gossypol**) is a possibility. One should attenuate with **hydroxycitrate** the excess of citrate-ATP citrate lyase activity, which feeds the formation of triglycerides, NADH the inhibitor of citrate synthase should increase, when one inhibits mitochondrial complex 1, (**capsaicin** for example). An inhibition of cytosolic Malate dehydrogenase with **D malate** would decrease this other OAA source. It is also useful to inhibit Phosphoenol pyruvate carboxykinase, with **chloro phosphoenol pyruvate**; or **cryogenin**. The latter may inhibit like hydrazine the GTP site of the enzyme, with less toxicity. It is crucial to inhibit transaminases **(aminooxacetic acid**, **β chloroalanine**) for ALA transamination. **DON** would inhibit glutaminase. Following the polyamine pathway, ornithine decarboxylase inhibition with **DMFO** deserves a try. Arginase ihibition with **norvaline** is easy to test; an activation of argininosuccinate synthetase with **troglitiazone** deserves a try. A **low arginine diet** is essential. One should favor the methylation of PP2A phosphatase with methyl donors (**Choline,** Vitamine **B12**, perhaps **trimethyl glycine**) methylated PP2A would dephosphorylate and activate Pyruvate kinase and Pyruvate dehydrogenase. It is essential to attenuate the choline kinase route with **miltefosine**, and the accumulation of triglycerides **metformin** might be useful (care not interfere with cryogenin) there are other possible drugs for controlling triglycerides.

Second part: physiological mechanism that lead to weight loss

We have seen that tumors burn their host; one should then block muscle proteolysis that is a rich source of amino acids, particularly alanine, which is vital to the tumor because it is the only amino acid transaminated to pyruvate, needed by the tumor because of the Pyruvate kinase bottleneck. Muscle proteolysis is the source of much ALA, we have already dealt with Alanine transaminase inhibition, and many compounds might be compared **aminooxacetic acid**, **hydroxylamine**, **pralidoxime (2 PAM)**. We have also dealt with glutaminase and arginase and explained that it is essential to inhibit ATP citrate lyase, which provides OAA to the transaminase cascade. Tumors absorb also fatty acids converted into Acetyl CoA; an intense lipolysis should be cancelled (**niacine**, **statins**).

Third part: epigenetic correction

A HDAC inhibitor should cancel the silencing of genes such as PETEN, P53 and others, one may use **butyrate** (**ammonaps**) or **vorinostat** the list of possible compounds is long. One may then try to control the cytochrome P450 demethylase, which might be involved in the demethylation of adjacent DNA regions, which recruit HAT and open other genes such as hexokinase. Inhibitors of cytochrome P450 such as **quinine** or **bergamottin** deserve a try. After some delay, one adds HAT inhibitors for closing other up-regulated genes (such as hexokinase) among these inhibitors one finds **anacardic acid**, **garcinol**, **curcumin** and others.

Fourth part: regulate signaling pathways

One should help with methyl donors the methylation of PP2A in order to target it toward the signaling kinases activated by insulin signals (**B12 choline or betaine**). As for the IGF signals elicited by Growth hormone (GH) **ocreotide** the somatostatin analog, or **pegvisoman** acting on IGF formation, will cancel the action of IGF. At the GH secretion level, **sulpiride** could be interesting. As for the IGFBP regulator of IGF, **apigenin** and **casodex**, seem to increase IGFBP, while NO decreases IGFBP. **Aminoguanidine** and other NO synthase inhibitors L- nitro arginine methyl ester (**L-NAME**), L-monomethyl arginine (**L-NMMA**), **agmatine** might block this undesired NO action, and reestablish an IGFBP control over mitosis.

Fifth part: the innate immune NK protection against tumor cells

Resistant cells should be eliminated by boosting the NK

protection, this is achieved by PGE2 inhibitors, such as **Echinacea extracts**, associated to **melatonine** or serotonine uptake inhibitors (**prozac**), or **St John Wort tea**, Substance P antagonists (**aprepitan**), inhibitors of indoleamine 2,3-dioxygenase(**1-methyltryptophane**) also boost the system. Moreover, HAT inhibition enhances the process (**anacardic** cashew extract, **garcinol**, **curcumin** etc)

Evidently, the duration of each phase should be determined; the different possible combinations of drugs tested on animal models. With the hope that a non-toxic mixture; will bring back to normality cells that have so easily taken the wrong pathway, at the detriment of the rest of the body.

B. Carcinogenesis (abridged hypothesis).

I. Oncogenes up regulate proteins transmitting "Tyrosine kinase receptor" signals. The receptor family includes those for insulin or IGF, a growth factor controlled by Growth Hormone. Other oncogenes alter PP2A phosphatase and remove the brake over the signaling kinases.
II. Experiments on pancreatectomized animals; treated with either pure insulin or total pacreatic extracts, showed that choline in the extract, preserved them from steatosis and hepatomas.
III. Since choline, is a methyle donor and since methylation regulates PP2A, it is probable that PP2A methylation protected from hepatomas, by attenuating signaling kinases; while the choline deficit lead to cancer.
IV. Demethylated PP2A would not deposphorylate Pyruvate kinase and Pyruvate dehydrogenases that remain inactivate. A"bottleneck" between glycolysis and oxidative-citrate cycle, interrupts the glycolytic pyruvate supply, now provided by alanine transamination and proteolysis. This pyruvate forms lactate (Warburg effect) and NAD+ needed for glycolysis. Lypolysis and fatty acids provide Acetyl-CoA; the citrate condensation increases; several oxaloacetate sources contribute. ATP citrate lyase follows, supporting aberrant transaminations with glutaminolysis and lipogenesis. Truncated urea cycles, an increased polyamine synthesis, consume the methyle donor SAM, strengthening carcinogenesis.
V. Epigenic changes triggered by a decreased butyrate, activates Histone deacetylase (PETEN, P53, IGFBP decrease; Hexokinase, fetal-genes-M2, increase)
VI. Normally, IGFBP binds IGF and controls mitosis, perhaps by capping; IGF receptors (IGFR) in a single daughter cell; in cancer, both cells would inherit IGFR and divide.
VII. An excess of IGF induces a decrease of the major histocompatibility complex MHC1, Natural killer lymphocytes should eliminate such cells that start the tumor; unless the fever prostaglandin PGE2 or inflammation, inhibits them...

In memory of Roger Marchbanks (1936-2010) a great biochemist

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