"Magische kugel" against cancer.

Yevgeny Tendler[1] Alexander Tendler [1*], Alexander Panshin[2]

[1]ExoProTher Medical Ltd., Haifa, Israel
[2]Kimron Veterinary Institute, Beit Dagan, Israel

Abstract

In the paper "Features of p53 protein distribution in the corneal epithelium and corneal tear film" a novel concept of a local anti-cancer defense mechanism was laid out. In this paper we further present and discuss evolutionary basis for its development and present perspective for its utilization as a novel therapeutic concept.

Keywords: Microvesicles, p53, Corneal epithelium, Tear film.

Accepted on 24 February, 2021

Introduction

Neoplastic processes are widespread in multicellular organisms give rise to malignant diseases that typical especially for all vertebrates. Signs of malignancy typically include six biological processes acquired during the multi-stage development of tumors:

1. Maintenance of proliferation signals
2. Avoidance of growth suppressors
3. Resistance to cell death
4. Replicative immortality
5. Induction of angiogenesis
6. Invasion and metastasis

These features are based on genome instability, which leads to the transformation of proto-oncogenes into oncogenes and a decrease or complete elimination of the tumor suppression function. As expected, both proto-oncogenes and tumor-suppressor genes were directly related to the emergence of multicellularity and the subsequent process of multilevel selection [1-4].

Study Description

It is noteworthy that almost all signs of malignancy, except for invasion and metastasis, are also inherent in unicellular organisms. The high instability of the genome gives them certain advantages in a changing environment, but also creates insurmountable difficulties in maintaining the viability of the species. Evolution made its choice, and instead of a highly variable RNA genome in almost all free-living organisms, the main carrier of heredity is a much more stable DNA genome. The basic principle of life for unicellular organisms is that: "I survive, and I don't care about the rest. In case of unfavorable conditions, I produce spores in order to preserve my unique gene pool".

With the transition to multicellularity, the basic principle of life has changed in 180 degrees. The life of an individual cell worth almost nothing, the main target is the whole organism prosperity. This required the creation of a new multilevel system of genomic and epigenomic regulation, to ensure the integration of an individual cell into a multicellular organism. Following control mechanisms should have been formed:

1. Control of proliferation
2. Regulation of differentiation,
3. Synchronization of metabolism,
4. Implementation of contact interaction with neighboring cells and tissues

Control of normality, including the possibility of destroying neighboring abnormal cells and even self-destruction.

Control of normality is the relevant for our discussion. During the formation of malignant tumors, mutated cells face fore types of antitumor defense:

1. Anti-tumor response within the cell itself
2. Anti-tumor agents originating from surrounding cells.
3. Reactions of innate immunity
4. Reactions of adaptive immunity.

The first gave rise to widely known and used therapeutic approaches such as radiation and chemo therapies. The last two are widely discussed in the literature as generally accepted “theory of immunoediting” [5], the development of the classical "theory of immunological surveillance" [6]. Based on this theory some features of the pathogenesis of malignant tumors have been explained and promising methods of immunotherapy treatments were proposed [7].

However, the elements of antitumor protection had to be formed before the appearance of special systems of immunological surveillance, otherwise multicellularity could not take place since it would be destroyed by selfish mutating cells. In the course of further evolution, these "antitumor" elements could continue to work independently or to be integrated into the systems of innate and adaptive immunity.

The second stage of local anti-tumor defense is an immediate extension of intracellular DNA damage repair and cell cycle regulation mechanisms. In this case, the anti-tumor defense is originating from neighboring cells. This direction has been much less studied. During evolutionary development of a multicellular organism, in parallel with the cell internal anti-oncogenic system, have formed systems for monitoring oncogenic changes in neighboring cells and, if necessary, destroying them. Delivery of tumor suppressors can be carried out both directly through intercellular contacts, and through secreted cellular vesicles, for example, exosomes.

The major player in cell anti-tumor response is the p53 tumor suppressor protein, also known as the "guardian of the genome" [8]. TP53 gene encodes the p53 protein, which controls the expression of proteins and their activity during cell cycle arrest, cellular senescence, DNA repair, and apoptosis [9]. It has been only recently discovered [7] that microvesicles containing p53 are actively secreted by corneal epithelial cells into the extracellular space and corneal tear film. They are re-captured by neighboring epithelial cells and play role in local corneal anti-cancer defense. Being an immune-privileged tissue, corneal epithelium lacks mechanisms of innate and adaptive immunity and has to fully rely on local anti-oncogenic functions. A tempting prospect arises to utilize these factors through novel therapeutic approaches.

In our recent experiments, we applied exosomes produced by corneal epithelial cells to human malignant cell line (LN18 glioblastoma (GBM)). Our results have shown significant anticancer activity of microvesicles preparations from chicken corneas in vitro. Furthermore, UV irradiation of corneal epithelial cells prior to microvesicles extraction significantly increased this effect indirectly supporting a hypothesis of p53 involvement (Figure 1).

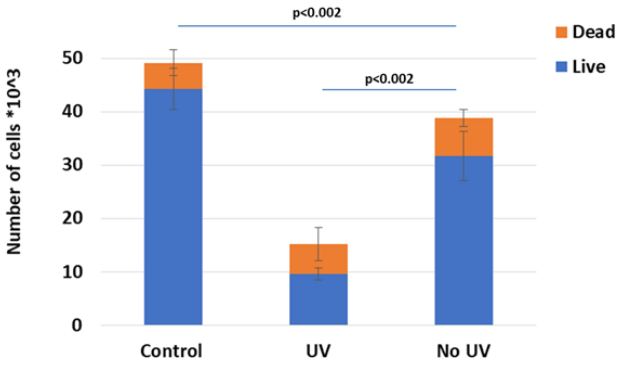

Figure 1. *Apoptosis assay 24h after addition of exosomes produced by chicken corneal epithelial cells to LN18 cell line (GBM p53 mut). No UV: Microvesicles harvested from chicken corneal epithelial cells without UV exposure; UV: Microvesicles harvested from chicken corneal epithelial cells following 5 min of UV exposure; Significant efficacy enhancement was achieved following UV irradiation, indirectly supporting p53 involvement hypothesis (ttest p<0.002).*

Methods

LN18 cell line 1 × 105 human GBM cancer LN18 cell line was seeded in 12 well plates in complete medium. 24 h later, the tested item or DMEM 0% FCS were added in triplicate. Following another 24 hours AnnexinV/ PI (Annexin V Apoptosis kit-MBL International) were detected according to the manufacturer’s protocol. Briefly, the cells were washed with PBS and then incubated in a solution of Annexin V and PI (diluted in annexin binding buffer). The cells were analyzed by flow cytometry and the results were analyzed with the FCS express program.

UV irradiation The cells were irradiated with a UV lamp (312 nm) at 150 mJ/cm2. The Petri dish was placed 15 cm under a UV light source (4 × 6 W, 312 nm tube, power 50 W, TFP-10 M, Vilber Lourmant, Torcy, France) for 5 min. The UV dosimetry was performed using a UV light meter (YK-34 UV; Lutron Electronic, Taiwan).

Discussion

In this paper we shared considerations directing development of novel anti-cancer therapy. However, there is one known obstacle on this path-in the process of oncogenesis, malignant cells acquire the ability to evade the action of their own antitumor mechanisms. Addition of wild type p53 protein to cells containing mutant p53 variant lead to their hetero-tetramerization and repression of wild-type p53 function [10].

In our opinion, orthologous genes encoding factors that carry out antitumor reactions of this type should be highly conservative and, have an anti-carcinogenic effect in xenogeneic organisms, at the same time, gene products in different species can differ significantly. Based on the current knowledge, it can be assumed that a protein similar

to the tumor-suppressor protein p53 is one of the factors exercising a surveillance function since the appearance of the first multicellular organisms [11]. It is appropriate to assume that drugs containing tumor suppressors of xenogenic cells will be able to suppress the growth of tumors that can escape from autologous anticancer factors. Due to the fact that, in some types of mutations, the mutant variant of this protein is able to suppress the activity of wild-type p53 a successful attempt was made to use the xenogenic chicken cornea as a source of a potential anticancer drug, since chicken p53 is resistant to the action of mammalian p53 mutant variants [12-16]. However, preparations from xenogenic cells are capable of causing a pronounced immune response, lowering their specific activity. Therefore, there is an urgent need to use a low immunogenic source. Since the anticancer activity can be associated with both components of cell membranes and cytoplasmic factors, the most appropriate carriers of activity can be cellular microvesicles containing membrane and cytoplasmic components.

Conclusion

In addition to the high content of p53, corneal cells can produce factors that interfere with some activities of cancer cells, namely: inhibition of vascular formation, prevention of invasion of neighboring cells, immunomodulatory properties and a finely balanced system of regulation of cell proliferation. Preparations from the cornea can contain a number of still unknown components with pronounced anticancer activity.

References

1. Loso DT, Tautz D. Phylostratigraphic tracking of cancer genes suggests a link to the emergence of multicellularity in metazoa. BMC Biology. 2010;8(66):1-10.
2. Rainey PB. Unity from conflict. Nature. 2007;446(7136):616.
3. Herron MD, Michod RE. Evolution of complexity in the volvocine algae: Transitions in individuality through darwin's eye. Evolution. 2007;62(2):436-51.
4. Pigliucci M, Okasha S. Evolution and the levels of selection. Biology & Philosophy. 2009;24(4): 551-60.
5. Dunn GP, Bruce AT, Ikeda H, et al. Cancer immunoediting: from immunosurveillance to tumor escape. Nature Immunology. 2002;3(11)991-8.
6. Burnet M. Cancer: A biological approach the processes of control. British Medical Journal. 1957;1(5022):779-86.
7. Egen JG, Ouyang W, Wu LC. Human anti-tumor immunity: Insights from immunotherapy clinical trials. Immunity Review. 2020;52(1):36-54.
8. Lane DP. p53, guardian of the genome. Nature. 1992;358:486-97.
9. Chen J. The cell-cycle arrest and apoptotic functions of p53 in tumor initiation and progression. Cold Spring Harb Perspect. 2016;6(3):1-16.
10. Willis A, Jung EJ, Wakefield T, et al. Mutant p53 exerts a dominant negative effect by preventing wild-type p53 from binding to the promoter of its target genes. Oncogene. 2004;23(13):2330-8.
11. Chumakov PM. Versatile functions of p53 protein in multicellular organisms. Biochemistry (Moscow). 2007;72(13):1399–1421.
12. Almazov VP, Morgunkova AA, Kalinin VN, et al. Construction of chimeric tumor suppressor p53 resistant to the dominant-negative interaction with p53 mutants. Molecular Biology. 2002;36(4):522–7.
13. Malkin D. Li-fraumeni syndrome. Genes Cancer. 2011;2(4): 475-84.
14. Kratz CP, Achatz MI, Brugières L, et al. Cancer screening recommendations for individuals with li-fraumeni syndrome. Clinical Cancer Research. 2017;23(11):38-45.
15. Tendler Y, Panshin A, Weisinger G, et al. Identification of cytoplasmic p53 protein in corneal epithelium of vertebrates. Experimental Eye Research. 2006;82(4):674-81.
16. Tendler Y, Panshin A. Features of p53 protein distribution in the corneal epithelium and corneal tear film. Sci Rep. 2020;10(1):10051.

***Correspondence to:**

Alexander Tendler

ExoProTher Medical Ltd.

Haifa

Israel

Tel: +972545696523

E-mail: alext@exoprother.com