

Euro Endocrinology 2018 : Alleviation of tamoxifen resistance by modulating $er\alpha$ alternative splicing - Kenji Ohe - Fukuoka University

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Abstract :

The estrogen receptor alpha ($ER\alpha$) gene has been extensively studied in breast cancer, however, the function as well as regulation of its splice variants are poorly understood. During our study of HMGA1a (formerly termed HMGI), which is known as a DNA-binding transcription factor, we found it regulates alternative splicing of $ER\alpha$ pre-mRNA as a sequence-specific RNA-binding protein. We searched for HMGA1a RNA-binding sites we previously identified and found one in $ER\alpha$ exon 5. HMGA1a binds to this sequence (detected by RNA-EMSA) that resulted in a splicing switch of two alternatively spliced isoforms, $ER\alpha66$ (full length) and $ER\alpha46$ (truncated). $ER\alpha46$ is application to improve tamoxifen effectiveness in breast cancer patients.

Introduction

oestrogen-receptor alpha ($ER\alpha$)-positive carcinoma. As Associate in Nursing adjuvant medical care in early carcinoma estrogen antagonist improves overall survival, and its widespread use is assumed to possess created a major contribution to the reduction in carcinoma mortality seen over the last decade (Early carcinoma Trialists' cooperative cluster 1998, Peto et al. 2000). In antecedently untreated pathological process carcinoma, quite five hundredth of patients with $ER\alpha$ -positive neoplasms win Associate in Nursing objective response or tumour stabilisation with estrogen antagonist (Lippman & Allegra 1980, Paridaens et al. 1980, Campbell et al. 1981, Stewart et al. 1982, Ingle et al. 1991, Jaiyesimi et al. 1995). estrogen antagonist may additionally have clinical utility as a preventative agent for hormone-dependent carcinoma (Cuzick et al. 2003). Despite the apparent advantages of estrogen antagonist in these treatment settings, the majority patients with metastatic sickness and as several as four-hundredth of patients receiving adjuvant estrogen antagonist eventually relapse and die from their sickness. The biological mechanisms underlying intrinsic (de novo) and bought resistance to estrogen antagonist area unit thus of appreciable clinical significance. higher understanding of those mechanisms could recommend novel methods to

known to inhibit AF-1 activity of $ER\alpha66$. Psoralen-mediated UV crosslinking showed HMGA1a anchored U1 snRNP to the adjacent pseudo-5' splice site. MCF-7 cells transfected with expression plasmids of HMGA1a and its RNA-decoy could induce and repress $ER\alpha46$ expression, respectively. The in vivo effect of the HMGA1a RNA-decoy were checked by transplanting its stable transfectants into nude mice, showing that they increased estrogen-dependent proliferation. In tamoxifen-resistant MCF-7 TAMR1 cells, the HMGA1a RNA-decoy improved tamoxifen-responsiveness by inhibiting estrogen-dependent cell proliferation. We conclude that this HMGA1a RNA-decoy would be implicated in novel therapeutic

The anti-oestrogen estrogen antagonist is that the most typically used treatment for patien

beat estrogen antagonist resistance and build any enhancements in carcinoma survival. Oestrogen receptor mechanism of action The target of estrogen antagonist in vivo is that the $ER\alpha$, and levels of $ER\alpha$ expression area unit the simplest predictor of take pleasure in estrogen antagonist (Early carcinoma Trialists' cooperative cluster 1998). Therefore understanding the biology of the $ER\alpha$ is crucial to understanding however resistance to estrogen antagonist could develop. The $ER\alpha$ could be a member of the nuclear receptor family of ligand-activated transcription factors. once getting into the cell, sex hormone binds the $ER\alpha$, that dissociates from heat shock proteins and undergoes conformational changes, phosphorylation and dimerization before binding to the sex hormone response parts (ERE) upstream of oestrogen-dependent genes. this can be said because the classical mode of action. $ER\alpha$ s also can regulate organic phenomenon while not interacting with desoxyribonucleic acid directly, via different transcription factors like the Fos/Jun activating protein-1 (AP-1) complicated (non-classical mode) (Kushner et al. 2000, Diamond State pepperwort et al. 2003, Schiff et al. 2003).

Recent knowledge additionally indicate that living substance membrane ER activity could act directly with key growth-factor dependent kinases, though the biological significance of those non-genomic effects of ER isn't totally understood.

The binding of ER to the ERE ends up in alterations in transcription of sex hormone regulated genes. Recent knowledge victimization microarray analysis to assess organic phenomenon within the MCF7 cell line, suggests that the bulk (70%) of oestrogen-regulated genes area unit down-regulated by treatment with estrogen (Frasor et al. 2003). Those genes down-regulated were shown to be transcriptional repressors, anti-proliferative and pro-apoptotic genes. Conversely there was up-regulation of positive proliferation regulators together with genes concerned in cell cycle progression (Frasor et al. 2003). wherever up-regulation will occur, this can be mediate by 2 distinct transactivation domains: Activating perform (AF)-1 (close to the N terminus) and AF-2 (in the ligand-binding domain). AF-1 activity is regulated by phosphorylation and is hormone-independent. AF-2 is integral to the ligand-binding domain (LBD) and thus is hormone-dependent. AF-1 and AF-2 act synergistically in most cells, however sure sequence promoters, generally as a results of their cellular context, are often severally transactivated by AF-1 or AF-2 alone (Gronemeyer 1991, dramatist et al. 2001). This promoter- or cell-dependence of ER-mediated transcription is also any modulated by the presence of co-regulatory proteins that area unit recruited to and act with promoter-bound receptor-ligand complexes. Co-activators that enhance transcription and co-repressors that suppress transcription have each been represented. Co-activators embody the p160 family that stimulate ER activity via interaction with AF-2. The 3 members area unit the nuclear-receptor co-activator one (NCoA1 additionally referred to as SRC1), NCoA2 (TIF2 or GRIP1) and NCoA3 (AIB1, TRAM1, RAC3 or ACTR)(McKenna et al. 1999, Leo & bird genus 2000). Different coactivators embody the SWI/SNF complexes, CREB-binding macromolecule (CBP), p300/CBP-associated issue (PCAF), and therefore the TRAP/DRIP/SMCC complicated (Chen et al. 1997, Sudarsanam & Winston 2000, Ito & Roeder 2001, Vo & Goodman 2001). These proteins accompany one another and therefore the general transcription machinery of the cell to

create giant complexes capable of synergistically activating oestrogen-driven transcription. several even have histone-acetyl-transferase (HAT) activity that ends up in body substance de-condensation and inflated rates of transcriptional initiation. 2 co-repressor proteins have additionally been described: nuclear receptor co-repressor one (NCoR1) and NCoR2 (also referred to as SMRT) (Chen & Evans 1995, Horlein et al. 1995, Heinzl et al. 1997, Nagy et al. 1997). Co-repressors influence transcription a minimum of partly by accomplishment of histone-deacetylase complexes (HDAC) that cause chromatin-condensation and decreased rates of transcriptional initiation. The matter certain ER complicated in association with co-regulatory proteins can't be regarded in isolation from different pathways within the cell. each the ER itself and co-regulatory proteins are often subject to phosphorylation at multiple sites by varied cellular kinases, raising the likelihood of any influences on ER-mediated transcription. once sex hormone binds to ER there area unit complicated interactions that result the last word influence of oestrogen-binding on transcription of oestrogen-responsive genes. Over recent years, knowledge has accumulated to recommend that in response to its matter, membrane- or cytoplasm-associated ER will act directly with key growth-factor dependent kinases (Kelly & Levin 2001). This non-genomic action happens apace following ligand-binding and is freelance of sequence transcription. the shape within which the ER mediates these non-genomic effects and its cellular localisation needs any study. but victimization Western blotting, ER α has been shown to be gift within the plasmalemmal caveolae (Kim et al. 1999), and in any studies a forty six kD ER splice-variant has been known, expressed within the cell wall (Li et al. 2003). Oestrogen-activated membrane ER α has been shown to activate the insulin-like growth factor-1 receptor (IGF-1R) cell signalling cascade by direct binding to IGF-1R, followed by its phosphorylation, a method that was found to be captivated with mitogenactivated macromolecule enzyme (MAPK) enzyme activity (Kahlert et al. 2000). additionally, matter activated membrane ER will phosphorylate and activate cuticular protein receptor (EGFR), and might act directly with variety of different key signalling molecules and pathways together with members of the Src family, matrix

metal loproteinases, G-proteins and therefore the regulative monetary unit of phosphatidyl-inositol-3 Buckeye State enzyme (PI3K) (Sun et al. 2001, Migliaccio et al. 2002, Wong et al. 2002, Razandi et al. 2003). though these effects area unit ab initio freelance of sequence transcription, activation of key secondary signalling pathways could mean subsequent effects on ER transcriptional activity area unit discovered (Sun et al. 2001, Wong et al. 2002). thus the genomic and non-genomic actions of the ER don't seem to be essentially freelance and beneath some circumstances could represent inter-related complementary pathways. When estrogen antagonist binds to the ER it ends up in completely different conformational changes to those discovered once sex hormone binds. sex hormone binds at intervals the hydrophobic pocket of the LBD and it's sealed within by helix twelve. the following conformational modification activates AF-2. In distinction, studies examining the crystal structure of the ER α LBD once sure to 4-hydroxytamoxifen recommend that beneath these circumstances helix twelve is prevented from waterproofing the binding pocket (Shiau et al. 1998). This positioning of helix twelve prevents the binding of co-activators and thence AF-2 transcription is prevented. thus {tamoxifen|estrogen Associate in Nursing|antagonist} inhibits AF-2 activation and functions as an antagonist on genes that consider the AF-2 region for ER-mediated transcription. However, in genes wherever AF-2 perform is redundant and transcription is driven by the AF-1 domain, {tamoxifen|estrogen Associate in Nursing|antagonist} could perform as an agonist (Tzukerman et al. 1994, McDonnell et al. 1995). The predominance of AF-1 or AF-2-activated genes specifically tissues (for example womb and breast respectively) has LED to the apparent tissue property of estrogen antagonist in its agonist and antagonist activity and therefore the derivation of the form SERM (selective sex hormone receptor modulator) to explain its mode of action. The potential for the agonist activity of estrogen antagonist to outweigh its antagonist effects has been a key focus of analysis on estrogen antagonist resistance. When estrogen antagonist is sure to the ER it additionally interacts with co-repressor molecules that lead to suppression of its agonist activity (Lavinsky et al. 1998). Therefore, altered convenience or accomplishment of co-regulators to the estrogen antagonist–

ER complicated in all probability contributes to the tissue dependence of tamoxifen effects, and will additionally underlie some estrogen antagonist resistance. The complexness of ER activation and estrogen antagonist interaction with ER provides many potential mechanisms by that resistance to estrogen antagonist would possibly evolve. Mechanisms of resistance Loss of ER α expression/function Since the results of estrogen antagonist area unit primarily mediate through the ER, and therefore the degree of ER expression could be a sturdy predictor of responses to estrogen antagonist, loss of ER expression may confer resistance to medical care. Indeed, lack of ER expression is that the dominant mechanism of Diamond State novo resistance to estrogen antagonist, with the bulk of ER/PgR negative tumours not responding to anti-oestrogens (Lippman & Allegra 1980, Paridaens et al. 1980, Campbell et al. 1981, Stewart et al. 1982, Ingle et al. 1991, Jaiyesimi et al. 1995). we've antecedently used Associate in Nursing immunohistochemical assay to see ER standing in seventy two paired biopsies taken before treatment and at progression or relapse on estrogen antagonist (Johnston et al. 1995). Patients whose tumours were treated with primary estrogen antagonist and responded before developing nonheritable resistance, often (but not always) remained ER-positive; sixteen out of eighteen (89%) were ER-positive pre-treatment, and eleven out of eighteen (61%) were positive on relapse. in a very second similar series, tissue microarrays were created from diagnostic assay samples taken pre-treatment and at relapse from patients treated with adjuvant estrogen antagonist. Of the twenty nine patients United Nations agency were ER-positive pre-treatment, 5 (17%) became ER-negative at relapse (Dowsett et al. 2003). Therefore, though ER expression is also lost in some patients United Nations agency develop nonheritable estrogen antagonist resistance, and be the mechanism of resistance in these patients, the bulk still categorical ER at progression. in truth up to twenty of patients United Nations agency have relapsed on estrogen antagonist answer aromatase inhibitors or to the ER-down-regulator fulvestrant suggesting that the ER continues to control growth even in several tamoxifen-resistant patients (Howell et al. 2002, dramatist et al. 2002). Co-repressors When co-repressors area unit recruited to the ER,

they kind multisubunit repressor complexes that embody HDACs, facilitating body substance condensation and inhibition of sequence transcription (Lavinsky et al. 1998, McKenna et al. 1999). The co-repressors area unit sometimes solely recruited once Associate in Nursing antagonist, like estrogen antagonist, is sure to the ER, and beneath these circumstances lead to a repression of its agonist activity. A study revealed by Lavinsky and coworkers confirmed that NCoR solely infirm associated with ER α within the absence of matter, however did thus avidly within the presence of the mixed anti-oestrogen trans-hydroxytamoxifen (Lavinsky et al. 1998). once NCoR activity was blocked victimization sublimata gamma globulin G against NCoR, trans-hydroxytamoxifen was born-again into Associate in Nursing agonist in MCF7 cells. In any studies MCF7 cells were deep-rooted into nude mice that were then treated with estrogen antagonist. NCoR levels (assayed on whole-cell extracts of the tumours) declined in several of the tumours that nonheritable resistance to the anti-proliferative effects of estrogen antagonist, relative to tumours holding a response to the drug. Taken along, these knowledge raise the likelihood that progressive reductions in co-repressor activity throughout estrogen antagonist medical care could enhance the agonist effects of estrogen antagonist on the ER contributive to resistance. in a very any clinical study there have been no variations within the levels of the co-repressor SMRT RNA in cohort of nineteen tamoxifen-resistant breast neoplasm samples, compared with twenty one untreated tumours however this study failed to assess NCoR levels (Chan et al. 1999). Cellular kinase/signal transduction pathways Oestrogen receptor biology can't be regarded in isolation from different animate thing signalling pathways. Over the previous couple of years, a substantial body of proof has emerged to recommend that there area unit multiple regulative interactions between the ER, protein and different enzyme signalling pathways. thence protein receptor pathways could up-regulate and stimulate growth severally of the ER, or may communicate via cross-talk with the ER and thereby have an effect on cell growth and patterns of resistance. Peptide protein signalling pathway Numerous studies counsel that cross-talk happens between ER and protein receptor

pathways, like the EGFR/HER2 family and insulin-like protein receptor (IGFR) family. The ER may be phosphorylated at the serine-118 position at intervals AF-1 by the MAPKs ERK1 and ERK2, that ar downstream parts of the HER2 signalling pathway (Fig. 2) (Kato et al. 1995). This enhances the sensitivity of the ER to matter and doubtless results in ligand-independent activation (Bunone et al. 1996). Serine-167 in AF-1 is additionally phosphorylated by a part of the kinase-mediated protein signalling pathway, during this case ribosomal S6 enzyme (RSK) that is itself activated by ERK1 and ERK2 (Joel et al. 1998). so hyperbolic ERK activity may doubtless contribute to resistance to endocrine medical care. Indeed, ERK1/2 expression and activity ar hyperbolic in many carcinoma cell-line models of endocrine resistance (Coutts & white potato 1998, Shim et al. 2000), and hyperbolic ERK 1/2 activity (assessed by phosphorylated MAPK immunostaining) correlates with shorter period of response to endocrine medical care in clinical carcinoma. The effects of the expansion issue signalling pathways on the ER ar bi-directional. once certain to matter, ER will activate protein receptors and their downstream kinases, and signalling molecules apparently by direct interaction at the cytomembrane (Kelly & Levin 2001). an immediate physical association between ER α and IGFR results in activation of IGFR and therefore the downstream ERK1/2 MAPK pathways (Kahlert et al. 2000). This interaction is blocked by the addition of the pure anti-oestrogen ICI 182,780 (fulvestrant) and by the MAPK substance atomic number 46 98059. Therefore, matter sure ER is capable of speedy activation of IGFR and its downstream signalling cascade. ER α additionally seems to act directly with HER2, and membrane-bound ER transactivates EGFR by phosphorylation (Chung et al. 2002, Razandi et al. 2003). Recent information additionally counsel that progesterin will act with the IGFR pathway by induction of internal secretion receptor substrate-2 (IRS-2) template RNA levels (Cui et al. 2003). short steroid hormone treatment was additionally found to extend binding of IRS-2 to Grb-2 and therefore the PI3K restrictive sub-unit p85, and result in increased ERK and AKT activation, demonstrating that cross-talk between endocrine and protein receptor pathways happens at many levels. Summary and conclusions Over the previous few years it's become apparent that

ER transcriptional effects aren't simply determined by the matter, however additionally by advanced interactions between co-regulatory molecules and multiple associated cell-signalling pathways. These

these signalling pathways and co-regulatory molecules are common accompaniments in experimental models of endocrine resistance. Rising clinical information suggests that such alterations may additionally play a section in resistance to estrogen antagonist during a clinical setting. Variety of medical specialty agents targeting these pathways are offered within the clinic or are in development. Additional confirmation in clinical samples that the pathways delineated are determinants of estrogen antagonist resistance might permit the evasion or hindrance of estrogen antagonist resistance victimisation rational therapeutic approaches. It'll be vital to contemplate that there is also multiple resistance pathways that are extremely vital in some patients however are tangential in others.

interactions give many plausible mechanisms by that carcinoma cells might circumvent a reliance on oestrogen-stimulated growth and become hormone-independent and tamoxifen-resistant. Altered levels of the expression of part

Biography :

Kenji Ohe has graduated from Kyushu University School of Medicine, Japan, with the specialties in Internal Medicine, especially Endocrinology. He completed his PhD at Kyushu University, Japan, and started working as a postdoctoral fellow at Paolo Sassone Corsi's lab when it was at IGBMC, France, and Akira Mayeda's lab when it was at Miami, FL, U.S.A. He is now working on therapeutic tactics on manipulating alternative splicing as an associate professor at Faculty of Pharmaceutical Sciences, Fukuoka University, Japan.

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