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Role of nuclear receptors in lung tumourigenesis

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Abstract

Nuclear receptors are a family of ligand dependent transcription factors which have important roles in control of growth and differentiation in many cell types. This review will focus on the role of two members of this family (estrogen receptors and PPARγ) in the initiation and progression of lung cancer. These receptors have been studied in other types of cancer, and the differences between findings in lung cancer and other malignancies will be discussed. We will also describe recent studies on the basic mechanisms of action of these receptors and suggest novel therapeutic targets. Specifically, there is emerging evidence that these receptors can induce gene expression through both ligand-dependent and ligand-independent pathways, and distinct families of genes are likely to be regulated depending on the mechanism of nuclear receptor signalling. These data suggest that a greater understanding of the basic biology and mechanisms of these receptors is required to develop specific pharmacological agents as therapeutics for cancer. Specifically, the development of selective agonists for these receptors, such as tamoxifen, may lead to more selective engagement of anti-tumourigenic pathways. In addition, combinatorial therapy using selective nuclear receptor activators in conjunction with the recently developed EGF receptor inhibitors (gefitinib, erlotinib) may sensitise cells which are unresponsive to the effects of EGF receptor inhibitors alone, providing powerful new therapeutic strategies.

Keywords: Nuclear receptors; Lung cancer; Peroxisome proliferator-activated receptors; Estrogen; Non-small cell lung cancer

1. Introduction

Nuclear receptors were first identified nearly 20 years ago by the isolation and cloning of the thyroid hormone receptor. During the past decade there has been an explosion of interest in these receptors, with the identification of the so-called "orphan receptors" (see [1] for review). From examination of the human genome there are currently 48 members of the nuclear receptor family (see NURSA web site: http://www.nursa.org). This family of proteins can generally be thought of as ligand-activated transcription factors, although recent studies have also demonstrated "ligand-independent" effects, which may be critical for their complex effects in cancer. From a structural examination, common features can be defined which are critical for DNA binding and activation of transcription. However, an important difference between the classical nuclear receptors and the "orphan receptors" is the nature and source of the ligand. In the case of classical nuclear receptors, the ligands are hormones which are produced largely through pathways derived from cholesterol biosynthesis. For most of the nuclear receptors however, the ligands have been less clearly defined. In fact, crystallographic examination of the ligand binding pocket of PPARγ shows a relatively large region, suggesting that a variety of ligands can occupy this site and signal transcriptional activation. These findings and other data have led to the model where these orphan receptors act as sensors for lipid products including eicosanoids, free fatty acids, and sterols. A great amount of interest has recently been focused on the role of these molecules in the cancer setting. The existence of

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pharmacological agents, including selective agonists and antagonists, which engage these receptors has made these molecules attractive candidates for therapeutic approaches. However, the biology of nuclear receptors is still not well understood. This review will focus on two specific classes of nuclear receptors, estrogen receptors, and members of the peroxisome proliferator activated receptors (PPARs). Much of the work in the area of cancer has focused on the effects of these receptors in breast and colon cancer, and many excellent reviews have been published in this area [2]. However, nuclear receptors in lung cancer have not been as intensely studied. Lung cancer is now the leading cause of cancer deaths in the United States, and novel therapies are desperately needed, since the overall 5 year survival is less than 5%. In addition, while studies in other cancers provide important clues as to the role of nuclear receptors in lung cancer, there are clear differences in the biology of the two organs, and care needs to be taken in extrapolating findings made in breast or colon cancer to carcinogenesis of the lung. In this review we will therefore focus on data examining the role of two members of the nuclear receptor family: PPARγ and estrogen receptors in non-small cell lung cancer. In addition to reviewing studies examining the biological effects of these receptors, we will also focus on recent work examining the molecular events associated with activation of nuclear receptors by ligands. These studies suggest novel targets for therapeutic intervention.

2. Biology of lung cancer

Lung cancer is the leading cause of cancer death for both men and women in the United States. In fact, more deaths will occur this year due to lung cancer than breast, prostate and colorectal cancers combined [3]. Lung cancers are categorised as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). As a group, the NSCLC constitute the bulk of lung cancers and are subdivided into squamous, adenocarcinoma and large cell carcinoma phenotypes. Selective changes in specific oncogenes can be used to distinguish the two types of cancer. Activating mutations in Ras are associated with NSCLC, with a mutation at codon 12 of the Ki-Ras gene observed in approximately 30% of adenocarcinomas, and just under 10% of other NSCLC types [4]. These mutations appear to be virtually absent from SCLC [5]. It should be emphasised that while Ras mutations are prevalent in NSCLC, they have been found with high degrees of frequency in other types of cancer, including pancreatic carcinoma, where the incidence rate is greater than 90%, and colon where the incidence rate is greater than 50%. In mice, K-ras mutations are found in over 90% of spontaneous and chemically induced lung tumours [6]. Overexpression of the c-myc gene is also frequently observed in NSCLC, but appears to be more prevalent in SCLC [7]. Elevated expression of the HER-2/neu gene, a member of the epidermal growth factor receptor family, has also been observed in 35% of adenocarcinomas and a slightly lower percentage of squamous carcinomas [8]. Alterations in tumour suppressor genes have also been reported. Mutations in p53 have been detected in 90% of SCLC and 50% of NSCLC [7]. Mutations in the retinoblastoma gene are more specific for SCLC, occurring in more than 90%, while only a small fraction of NSCLC have mutations in this gene. Recently, mRNA expression profiling has been used to define subclasses of lung adenocarcinoma. These studies employed microarray technology to define patterns of gene expression characteristic of these subclasses [9,10]. Interestingly, one of these studies [10] defined a subgroup of adenocarcinoma characterised by increased expression of a number of enzymes in the eicosanoid pathway. Patients in this group had a significantly lower survival rate, and high incidence of metastases. In contrast to most NSCLC, SCLC displays neuroendocrine features exemplified by the presence of cytoplasmic neurosecretory granules containing a wide variety of mitogenic neuropeptides including gastrin-releasing peptide, arginine vasopressin, neurotensin, cholecystokinin and many others [11,12]. Significantly, SCLC also expresses G protein-coupled receptors for these neuropeptides, thereby establishing autocrine-stimulated cell growth.

There has been a great deal of work examining the role of receptor tyrosine kinase inhibitors as therapeutic agents. EGF receptor inhibitors (gefitinib and erlotinib) have been shown to have beneficial effects in a subset of lung cancer patients [13,14]. In fact, somatic mutations in the EGF receptor have been identified in many of these patients which sensitises the cancer cells to growth inhibition by these agents [15]. Unfortunately, the majority of smokers are resistant to these agents, and an inverse correlation with oncogenic mutations in K-Ras has been observed. A major challenge therefore remains to develop approaches that can sensitise cells to EGF receptor blockers. Nuclear receptors are attractive candidates for these approaches, with the possibility that combination therapy using targeted nuclear receptor activators or inhibitors and EGF-receptor blockers will provide effective treatment for a significant subset of lung cancer patients.

3. PPARγ receptors and cancer

PPARs are members of the nuclear receptor superfamily, and were initially demonstrated to have important roles in lipid metabolism (see [16] for review). The three PPAR isoforms, α, γ, and δ (also known designated β) bind as heterodimers with the retinoic acid X receptor (RXR) to specific regulatory promoter elements (PPAR-RE). The consensus element for PPARs is a direct repeat of a six nucleotide sequence, with a sin-

gle intervening base (AGGTCA*N*AGGTCA). PPARγ has been cloned from a number of species including human [17]. In both mice and humans, two isoforms of the protein are expressed PPARγ1 and PPARγ2, which are alternate splice variants. PPARγ2 has additional 30 amino acids, and its expression is largely restricted to adipose tissue, whereas PPARγ1 is widely expressed [16]. PPARγ is activated by polyunsaturated fatty acids and eicosanoids. In particular, 15-deoxy-$\Delta^{12,14}$-PGJ_2($dPGJ_2$) has been shown to specifically activate PPARγ with micromolar affinity [18]. Lipoxygenase products of linoleic acid, 9- and 13-HODE have micromolar affinities for PPARγ [19]. It is not clear whether any of these agents are actual physiologic regulators of PPARγ, and a recent study has found that endogenous levels of $dPGJ_2$ do not change during adipocyte differentiation [20]. Synthetic activators of PPARγ include the thiazolidinediones (TZDs), such as ciglitazone and troglitazone [21]. These compounds have insulin-sensitising and anti-diabetic activity, which is likely mediated at least in part through PPARγ activation. Finally non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit eicosanoid production, activate PPARγ albeit at higher concentrations than required for COX inhibition [22].

A critical role for PPARγ has been established in adipocyte differentiation, where activation leads to induction of adipocyte-specific proteins including aP2 [23]. Homozygous deletion of PPARγ is embryolethal; heterozygotes have reduced amount of adipose tissue [24,25]. In adipocytes, PPARγ regulates genes involved in lipid metabolism and energy homeostasis. Importantly, activation of PPARγ also regulates genes involved in insulin action, which results in cells being sensitised to insulin. This has led to the use of PPARγ activators (see above) as anti-diabetic agents. PPARγ activation has also been implicated as a regulator of processes associated with atherosclerosis. Targeted deletion of PPARγ demonstrated a critical role for PPARγ in cholesterol metabolism [26]. This is most likely to be mediated through induction of the cholesterol transporter known as ABC-1.

Activation of PPARγ has been implicated in many types of cancer. Consistent with a role in adipocyte differentiation, PPARγ activators (troglitazone) inhibited growth and led to induction of differentiation in liposarcoma [27]. A large number of studies have examined the role of PPARγ in colon cancer, with apparently conflicting data. TZDs inhibit the growth of colon cancer cell lines *in vitro*, and in xenograft models [28]. Inactivating mutations have also been reported in colon cancers [29,30], suggesting that PPARγ may act as a tumour suppressor gene. Mice with one copy of the PPARγ gene(+/−) have an increased tendency to develop colon tumours compared to wild-type littermates when exposed to azoxymethane. However, administration of PPARγ activators to APC^{Min} mice, which have an inactivating mutation in the adenomatous polyposis Coli (APC) gene, resulted in increased tumour numbers [31,32]. This apparent conflicting role may be due to alterations in the normal differentiation of colonic epithelial cells which occurs in the APC^{Min} mouse. These mice have increased expression of β-catenin in the colon, compared to normal colonic epithelia, which downregulate β-catenin during differentiation. TZDs have also been shown to inhibit the growth of breast cancer [33] and prostate cancer cells [34]. However, mice with targeted expression of a constitutively active form of PPARγ in the mammary gland when crossed to transgenic mice prone to develop breast cancer, resulted in tumours with increased rates of growth [35]. This may be a consequence of increased signalling through the Wnt pathway, suggesting that PPARγ may serve as a tumour promoter. A potential explanation for these conflicting data may the nature of the mouse model (see below). Nevertheless, the fact that large numbers of women are exposed to TZDs as anti-diabetic agents, requires additional definitive studies on the role of PPARγ in breast cancer.

3.1. PPARγ in lung cancer

The role of PPARs in the development of lung cancer has not been studied as extensively as in colon or breast cancer. Several studies have demonstrated that activation of PPARγ can inhibit growth of lung cancer cells [36–38]. These studies treated either lung cancer cell lines or xenograft models with TZDs. Pharmacological agents led to growth arrest and induction of apoptosis in cell lines, and inhibition of tumour growth in xenograft models [39]. In samples from human lung tumours, decreased expression of PPARγ has been correlated with poor prognosis [40]. In a separate study expression of PPARγ was examined by immunohistochemistry. Adenocarcinomas that were differentiated very well had greater frequency of PPARγ-positive cells than poorly differentiated samples. Exposure of NSCLC cell lines to PPARγ activators has been shown to regulate expression of proteins which may mediate lung cancer cell growth, including upregulation of p21 [41] and downregulation of PGE_2 receptors [42]. Our laboratory has applied a molecular approach to examine the effects of PPARγ in NSCLC. In contrast to findings using pharmacological activators, we have observed no effect on cell growth in standard culture [38]. However, increased PPARγ activity through overexpression resulted in enhanced differentiation of the cells and impaired anchorage-independent growth and invasiveness [43]. This has also been reported by other workers [36].

It is important to develop an understanding of the underlying mechanisms contributing to different effects observed by different investigators. A complication in assessing the role of PPAR activators is emerging data

that these agents engage additional targets that may mediate some of their effects on tumourigenesis. Induction of apoptosis of gastric epithelial cells by 15-deoxy-$\Delta^{12,14}$-PGJ_2 occurs in a PPARγ-independent fashion, and involves activation of the JNK family of MAP kinases [44]. Troglitazone can induce expression of the immediate early gene egr-1 through a PPAR-independent mechanism [45]. In addition, different ligands for PPARγ recruit different co-activators, resulting in ligand-specific changes in gene expression. In macrophages derived from PPARγ knockout mice, both TZDs and $dPGJ_2$ inhibited release of cytokines to the same extent as in wild-type macrophages [46]. PPARγ activators also inhibited growth of ES cells from PPARγ null mice via a mechanism that involves inhibition of translation [47]. Activation of NF-κB by $dPGJ_2$ is also observed in PPARγ-deficient cells [48]. NSAIDs, which activate PPARγ also inhibit COX isoforms, and eicosanoids, which can activate both PPARγ and PPARδ also signal through cell surface receptors coupled to G-proteins [49]. Thus care must be taken in attributing therapeutic effects of putative PPAR regulators to activation of specific PPARs. There are also limitations in the use of molecular approaches employing overexpression. Saez and coworkers [35] have studied mice with targeted overexpression of a constitutively active form of PPARγ, in which the VP16 activation domain is fused to the PPARγ DNA binding domain. This fusion protein is presumably constitutively active, and has lost the regulated activity of wild-type PPARγ in response to ligands. It is therefore conceivable that subsets of genes will show altered expression that does not occur in the setting of wild-type PPARγ. Finally, there is evidence that PPARγ can signal through ligand-independent pathways. The co-activator PGC-1 binds to the hinge region of PPAR in a ligand-independent fashion. The co-activator p300 can bind in a ligand-dependent and ligand-independent fashion to PPARγ [50].

3.2. Mechanism of action of PPARγ

Functional domains of PPARγ have been defined, which are present in other members of the nuclear receptor superfamily (see Fig. 1A). These include a DNA-binding domain which is conserved in all three isoforms [16]. A ligand binding domain is located in the C-terminal half of the molecule; crystallographic studies have shown that the ligand binding pocket is larger than for other nuclear receptors, allowing these molecules to interact with a variety of activators [51]. A ligand-dependent activation domain, designated AF-2 is located in the C-terminal region and enables binding of co-activators. A ligand-independent activation domain, AF-1 is located in the N-terminal region. Ligand binding to PPAR causes a conformational change in the molecule which results in recruitment of co-activators such as

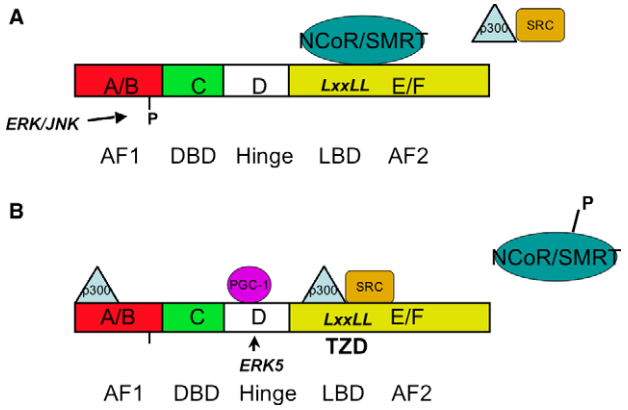

Fig. 1. *Modulation of PPARγ*. The structural domains of PPARγ, which are conserved among members of the nuclear receptor superfamily are indicated. (A) Under basal conditions, PPARγ transcriptional activity is repressed by binding of co-repressors (NCor/SMRT). Phosphorylation by members of the MAP kinase family (ERK/JNK) also inhibits activity. (B) Activation is mediated through ligand (TZD) binding. Phosphorylation and degradation of co-repressors leads to binding of co-activators (SRC, p300). Some co-activators (PGC-1) can bind in a ligand-independent fashion. Activation through the ERK-5 pathway binding to the hinge region of PPARγ has also been reported.

p300 and SRC-1, leading to increased transcription of target genes. This is associated with displacement of co-repressors, specifically SMRT and N-CoR. Components of this co-repressor complex serve as adapters for the ubiquitin conjugatin/19S proteosome complex [52], resulting in dissociation and degradation of co-repressors. Agonists may differ in the extent of the conformational change, resulting in differential abilities to recruit these co-activators, and ultimately differences in the ability to induce specific genes. In addition, ligand independent binding of co-activators has been reported which may result in PPAR activity in the absence of ligand binding. Specifically, the co-activator p300 can bind to both the AF1 region in a ligand-independent fashion, and to the AF2 region in a ligand-dependent fashion, mediating transcriptional induction under both conditions [50]. Regulation of PPARγ also is mediated through MAPK pathways. Phosphorylation of serine residues in the amino terminal by ERK or JNK members of the MAPK family is associated with inhibition [53,54]. This is likely mediated through changes in the affinity of activating ligands [55]. Interestingly, ERK5, another member of the MAPK family has been reported to activate PPARγ in a ligand-independent fashion, by binding to the hinge region of the molecule [56].

4. Estrogen receptors in lung cancer

Estrogens and their receptors are important in the regulation of cell growth, differentiation, and the physiology of the reproductive system through the estrogen

receptor [57,58]. The estrogen receptor α (ERα) regulates transcription of various estrogen target genes and is best known for its association with the development of estrogen-dependent cancers such as breast and endometrial cell cancers [59,60]. Estrogen is also a recognised risk factor for lung cancer in women [61]. There is evidence that both exogenous and endogenous estrogens may play a role in the development of lung cancer, especially, adenocarcinoma, among women [61]. Interestingly, ERα does not appear to play a significant role in the advancement of NSCLC [59,61]. However, the expression of ERβ in human lung cancers has been identified by several studies as playing an essential role in the development of NSCLC [59,62].

4.1. The characteristics of the estrogens receptors α and β

The estrogens are members of the nuclear receptor superfamily of transcription factors. The ERα gene is located on chromosome 6 and was the first estrogen receptor cloned [2]. The ERβ was subsequently cloned fifteen years later and is located on chromosome 14 [2]. The difference in chromosome position suggests that both ERα and ERβ are indeed encoded by separate and distinct genes. ERβ has similarities to ERα in terms of its structure and function. Both ERα and ERβ share common structural domains (see Fig. 1B). The designated activation function 1 (AF1), however, is the least conserved domain between ERα and ERβ. In fact, ERβ has been shown to lack AF1 activity. As for PPARs, AF1 is associated with ligand independent activation, where as the AF2 region has a ligand dependent activation function. Other regions, such as the DNA binding domain C, are well conserved between the two, whereas the D domain, *i.e.*, the hinge region, is not well conserved. The E/F regions, or ligand binding domain encompasses a co-regulator binding surface, the dimerisation domain, a second nuclear localisation signal, and the AF2. Both ERα and ERβ have similar affinity for estrogen, but differences have been seen for other ligands. In addition, ERβ and ERα also differs in their tissue distribution, and although ERβ is seen in breast, relative levels of ERβ mRNA expression is highest in human granulosa cells, endothelial cells, ovary, and lung [2,63].

4.2. Ligand-dependent versus ligand-independent ER transcriptional activity

The classical scheme of ER activation involves estrogen ligand dependent binding to the ER, the subsequent dissociation of the heat-shock proteins from the ER and receptor dimerisation. The ER dimer then interacts with co-regulatory proteins, binds to DNA sequences termed estrogen response elements (ERE) that are located in the regulatory regions of responsive target genes, and transcription is activated [64]. Signalling through ER can also occur as a result of ligand-independent activity. Cross talk between the ER and other signalling pathways are responsible for the activation of this ligand-independent transcriptional activity [65]. ER is most commonly phosphorylated on serine residues in the AF1 region [2,65]. MAP kinase appears to play an important role in the phosphorylation of the serine residues in the AF1 region of ER [2]. In addition, the phosphatidylinositol-3-OH kinase (PI3K/ Akt), EGF, IGF, cyclinA-CDK2, casein kinase II, pathways also appear to be important in serine phosphorylation of the AF1 region in ER [2,64]. Due to the well known association of growth factor signalling and the ER, it is important to note the activation of ERα by phosphorylation at multiple serine sites by multiple kinases [64]. In fact, growth factor signalling may account for the loss of estrogen dependence resulting in the creation of anti-estrogen resistant tumours [66]. Furthermore, it has been shown that increased MAP kinase phosphorylation and activity is associated with a worse response to endocrine therapy in some cancer patients [2]. Of note, since the AF1 region is not present in ERβ, it is unlikely that these ligand independent factors will play a significant role in ERβ signalling. Little is known about the phosphorylation of ERβ. However, it is known that the phosphorylation of the unliganded receptor by MAP kinase in the AF1 region increases the recruitment of SRC-1 and thus possibly the activation of ERβ [64,65]. The importance of ER phosphorylation and clinical outcomes however, continues to be largely unknown.

4.3. Non-canonical ER transcriptional activity

ER is also able activate transcription by tethered interactions through protein–protein interactions at both the AP1 and Sp1 sites [2]. Both ERα and ERβ have been shown to stimulate the AP1 reporter [67]. However, not surprisingly, both Erα and ERβ respond differently to estrogens and anti-estrogens at the AP1 sites [67]. The ER also activates transcription of downstream target genes by way of the Sp1 site [2,65]. Both ERα and ERβ have been shown to bind to the Sp1 protein [64]. There are a list of estrogen responsive genes that show activated transcription through non-consenus ERE sites. These genes include: c-Myc, cathepsin D, heat shock protein 27, creatine kinase, and TGFα [2].

4.4. Effects of co-regulators on ER function

Transcriptional activation by ER requires the recruitment of many regulators, such as co-activators, co-repressors, co-integrators, histone acetyltransferases, and histone deacetylases. These components of regulation all interact to influence the transcription and accessibility of target gene promoters. Co-activator proteins augment transcription. Most co-activators involved in

the interaction of ER are associated with the LXXLL motif [68]. The co-activators of ER include steroid receptor co-activator 1 (SRC1), SRC-2, and SRC-3 [64]. Co-repressors reduce transcription and are comprised of nuclear receptor co-repressor (NCoR), and silencing mediator for retinoid and thyroid hormone receptor (SMRT) [62]. The co-integrators include p300 and CREB-binding protein. The co-integrators, unlike the co-activators and co-repressors do not directly bind to DNA, but are recruited to promoter sites by other transcription factors. The co-regulators provide for another layer of control of ER transcriptional activity, and in general they interact with multiple members of the nuclear receptor family [65]; however few co-regulators can interact with only ER [62]. Some co-activators have been shown to be very selective in the interaction with either ERα or ERβ. One such example is the with SRC-3, which enhances ERE transcriptional activity, but has no effect on ERβ mediated transcription [61]. Co-regulators affect the remodeling of chromatin to allow for gene transcription to occur [69]. Histone acetylation is associated with transcription, whereas, deacetylation is correlated with repression of gene transcription.

4.5. ERa and ERβ expression and function in non small cell lung cancer

ER clearly has a role in multiple cancers. Most notably, breast carcinomas where ERα plays a particularly important role in the development of breast cancers [2]. The majority of ER present in breast tumours is ERα. In fact, the vast majority of targeted therapy has been focused on disrupting the interaction between estrogen and ERα. Interestingly, some of the therapies for breast cancer, for example SERMs (*i.e.*, tamoxifen, raloxifene) which are competitive inhibitors of estrogen activation, can not only act as estrogens in various tissue, but can also act as anti-estrogen in other tissue [65]. ERα also plays an important role in the development of ovarian cancer with almost 67% of ovarian cancers being ER positive [65]. Several studies have not demonstrated an essential role of ERα in NSCLC development [61].

By contrast ERβ does not appear to play a significant role in the development of breast carcinomas [2]. In fact, in breast cancer the presence of ERβ may indicate a good prognostic marker, and the loss of ERβ maybe critical for the development of tumourigenesis in normal breast tissue. In the lung however, ERβ plays an important role in the development of NSCLC, and is the predominant form of ER expressed in the lung [61]. Interestingly, because ERβ is the predominant ER type in the lung, the mechanism is primarily a ligand dependent estrogenic response in NSCLC [60,70]. It has been shown that using a pure estrogen antagonist ICI 182,720 (*i.e.*, fulvestrant or faslodex) reduces the cellular proliferation of NSCLC cells by way of blocking estrogen ability to signal through ERβ. The predominance of ERβ is a potentially important therapeutic target in NSCLC.

It appears that both ERα and ERβ signal in different ways depending on the ligand and the response element, suggesting that both ERs play important but different roles in gene regulation. The differences in ERα and ERβ expression in various tissues (*i.e.*, breast verses lung) may in part explain the reason why ERβ acts as a tumour suppressor in breast carcinoma, but behaves as a ligand dependent estrogenic promoter of tumour growth in NSCLC. Targeting the ERβ by way of employing pure anti-estrogen agents in NSCLC may serve as novel therapy for the treatment of NSCLC of adenocarcinoma origin.

5. Conclusion

Nuclear receptors represent attractive targets for novel therapeutic approaches in lung cancer. One attractive aspect of studying the effects of both estrogen receptors and PPARγ is the existence of well-characterised pharmacological agents which activate these receptors. Secondly, the effects of activators of these receptors can potentially modify the state of differentiation of lung tumours. Both ER agonists and PPARγ ligands modulate expression of E-cadherin in NSCLC [70,71], which is a marker for the epithelial state of lung cancer cells. It has recently been reported that increased levels of E-cadherin is also a marker for sensitivity of NSCLC to gefitinib (Witta, S personal communication). Therefore, we would propose that exposure of NSCLC to selective nuclear receptor agonists may independently modulate their sensitivity to EGF receptor inhibitors (Fig. 2). Combinatorial therapy employing nuclear receptor activators in conjunction with EGF receptor

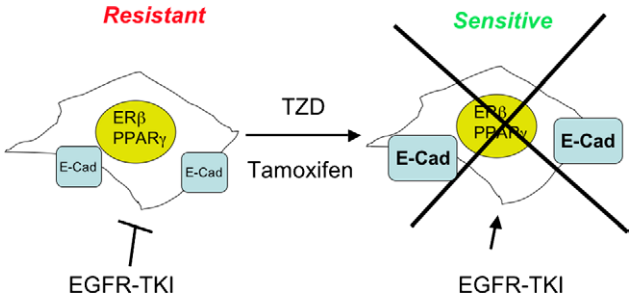

Fig. 2. *Sensitisation of NSCLC to EGFR inhibitors.* The majority of lung cancer cell lines and tumours are resistant to the effects of EGF receptor tyrosine kinase inhibitors (gefitinib, erolitinib). However, it is postulated that in the presence of activators of nuclear receptors such as ERβ and/or PPARγ these cells become sensitised to the effects of these agents, in part through increased differentiation, reflected as up-regulation of E-cadherin. Combination therapy of nuclear receptors activators and EGFR inhibitors presents a novel therapeutic approach.

blockers provides an attractive strategy in treating EGF-receptor inhibitor resistant patients, which constitute the majority of lung cancer cases. Finally, the recognition that the ubiquitin/proteosome pathway is critical for activation of nuclear receptors [72], suggests that nuclear receptors may be critical targets for proteosome inhibitors, whose role in lung cancer is currently under active investigation.

Conflict of interest statement

None declared.

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