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EGF receptor as a therapeutic target

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Summary EGFR and Her-2 are overexpressed in lung cancers and have, therefore, been chosen as preferred targets for novel therapies. Recent results on Iressa trials show only a moderate response to the agent, even in cases where it is documented that EGFR is over-expressed. These findings prompted us to re-visit the oncogenic pathways, which play a role in lung cancers, with special emphasis on the way EGFR/Her-2 signaling cooperates with other signaling pathways.

1. Introduction

EGF receptor and its ligands are involved in over 70% of all cancers. Due to its overexpression, co-expression of the receptor, and its ligands, as well as activating mutations, the enhanced activity of the receptor is the hallmark of many human carcinomas [1]. In many types of tumors, including lung, breast, prostate, ovary, gastrointestinal tract and brain, the EGFR receptor is expressed approximately 100-times more than the normal number of EGF receptors found on the surface of the normal cell. Overexpression of at least two of these receptors, the EGF receptor (EGFR) and the closely related ErbB2, has been associated with a more aggressive clinical behavior [2]. Indeed, expression of high levels of these two receptors in nonmalignant cell lines leads to a transformed phenotype. There is no surprise, therefore, that EGFR was identified early on as an important target for drug development [3,1,4,5]. A number of therapeutic strategies specifically target either the inside- or the outside of the EGF receptor and its family members in order to nullify their oncogenic activity (Fig. 1).

2. Modes of EGFR activation

The enhanced activity of the EGFR is due to a number of molecular events, which can lead to the persistent activation of the kinase activity of the receptor. Most common is the overexpression of the receptor along with the expression of the EGFR receptor ligands like TGFα, EGF, amphiregulin and HB-EGF leading to persistent autocrine stimulation. Another common occurrence is the activating mutation where the exons 2–7 are deleted, leading to a persistently active receptor EGFRΔ (2–7), in the absence of a ligand [6,7]. This activating mutation is the hallmark of many tumors that overexpress EGFR. The emergence of this mutation represents the most aggressive form of the tumor. In many cases EGFR is co-expressed with other members of the EGFR, leading to the formation of highly transforming dimers such as EGFR–ErbB2 and EGFR–Erb3. It is well known also that other dimers such as ErbB2–ErbB3 play a key role in various cancers such as breast cancer.

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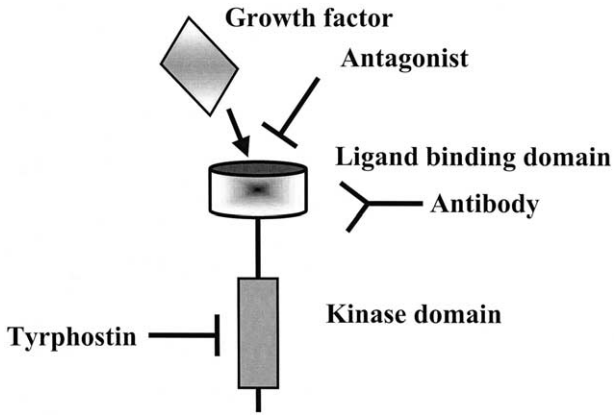

Fig. 1 Modes of EGFR inhibition. Two domains in the EGFR can be targeted: the ligand binding domain and the catalytic domain. Antibodies to the EGFR and low molecular weight catalytic inhibitors are two strategies, which have shown promise.

3. EGFR transactivation

Activation of the EGFR pathway is not limited to members of the EGFR family but can occur by the cross talk with other signaling pathways such as mitogenic G protein coupled receptors [8] and PDGF receptor [9]. Furthermore, the EGFR pathway cooperates in a synergistic manner with $pp60^{c\text{-}Src}$ [10], Jak-2 and the deletion of PTEN (see below). Thus targeting the EGFR for therapy is not limited to cases in which the EGFR pathway is activated directly but also to cases where the EGFR is an important component of the complex oncogenic network. EGFR receptor is activated by G protein coupled receptors by a process known as transactivation [11]. A number of mitogenic G protein coupled receptors have been shown to induce the release of a heparin bound EGF (HB-EGF) receptor ligand by the activation of a proteolytic enzyme. The HB-EGF precursor and metalloproteinase activity were found to be critical pathway elements between GPCR signals and the activation of the EGFR [11–13]. This form of EGFR transactivation represents a widespread mechanism by which the activation of heterotrimeric G-proteins by interaction with a GTP binding protein coupled receptor (GPCR) results in an intracellular signal that induces the extra cellular activity of a transmembrane metalloproteinase. This activity is inhibited by Batimastat and is specific for the interaction between a GPCR and the EGFR. This pathway is used by a variety of GPCRs in diverse cell types and seems to play a key role in the mitogenic signals in a number of cancer cells. The pathophysiological relevance of this mechanism for the various forms of lung cancer is yet to be assessed. It seems that if it will be discovered that the EGFR family is transactivated in lung cancers, as it does in other cancers [8], additional therapeutic agents such as metaloproteinase inhibitors may be considered.

4. Modes of EGFR/Her-2 blockade

Since EGFR and Her-2 seem to play an important role in the pathology of a significant portion of lung cancers clinicians have targeted them. Three approaches can be used to target the receptor itself: (1) the design and synthesis of ligand antagonists, (2) the utilization of antibodies, which induce receptor inactivation like Herceptin for Her-2 and monoclonal antibody 225 against the EGFR and (3) tyrphostins (tyrosine phosphorylation inhibitors, tyrosine kinase inhibitors) which block the kinase activity of the receptors. Approaches (2) and (3) have gained momentum whereas the attempts to generate small molecules which block the ligand binding domain (approach 1) have not met with success and have largely been discontinued. The antibodies ABX-EGF [14,15] and Erbitux [16] bind to the extra-cellular domain of the receptor paralyzing its activation by its ligands. Along with Gleevec and Herceptin these are the first successful signal transduction inhibitors whose design is based on the emerging knowledge pathways in the signaling of cancer cells [17].

5. EGFR and Her-2 directed kinase inhibitors

The blocking of EGFR kinase activity has been shown to block EGFR driven tumors both in cell culture and in vivo. A number of drugs targeting the kinase activity of the EGF receptor are being developed, including ZD1839 (Iressa®)[18,19], already approved, and OSI-774 (Tarceva™) [20], which is in clinical trials. Both are orally active. Another quinazoline in clinical development is AG 1478 [21,22], which will be administered to patients with glioblastoma multiforme intravenously. These clinical trials are conducted by the Ludwig Institute for Cancer Research (LICR) in Melbourne, in collaboration with the Hebrew University of Jerusalem. Iressa and Tarceva are inhibitors of tyrosine phosphorylation, blocking the catalytic activation of the receptor, namely its activation as well as its ability to phosphorylate external substrates. Interestingly, Iressa was found to be moderately successful as a single agent for lung

cancer but did not synergize with CDDP [23]. These results probably reflect the fact that in the patients targeted, EGFR was not a survival element on which the cancer depends and, therefore, inhibition of EGFR was not too effective. Furthermore, it is likely that in many patients, who suffer from lung cancer, EGFR is not playing an important role in the disease and the tumor may not even over-express the EGFR. Over-expression is likely to be an indication for the important role the receptor may play in the tumor. Interestingly, in an animal model of human glioblastoma multiforme in which EGFRΔ (2–7) is over-expressed we could show inhibition of receptor kinase activity in vivo only when the EGFR directed tyrphostin AG 1478 was applied along with CDDP [22]. We can tentatively conclude from these observations that an EGFR kinase inhibitor will be effective only when (1) EGF/EGFR signaling is essential for tumor survival and growth and (2) that the agent applied needs to block effectively the kinase activity in vivo for a significant length of time during application. Recent work suggests that irreversible kinase inhibitors of EGFR may be superior in achieving this goal. The main problem with irreversible inhibitors such as CI-1033 [24] is their toxicity (see [25]). This is most probably due to the high chemical reactivity of the acryloyl group that is responsible for its irreversible nature. Recognizing this deficiency we synthesized an irreversible EGFR kinase inhibitor, MLO5, which possesses very high affinity towards the EGFR ($IC_{50} = 37$ pM) (Aburbeh et al., unpublished) and much more moderate reactivity of the chemical group attached to the quinazoline pharmacophore. This combination of properties leads to a longer residence of the compound within the EGFR kinase domain such that it allows for a reaction to occur between the moderated alkylating group and the EGFR cysteine 773. On the other hand the moderated chemical reactivity of MLO5 as compared with CI 1033, enables it to be washed off from the low affinity non-specific sites before it has a chance to label them as CI 1033 does. We are now testing the validity of these findings in tissue culture systems as well as in an animal model. These experiments are designed to examine whether indeed MLO5 is less toxic than CI 1033 but retains a strong anti-tumor activity. If we find that MLO5 is indeed less toxic, we shall consider developing it for clinical trials.

Frequently, the EGFR hetero-dimerizes with other members of the EGFR family, especially Her-2; this is also true for lung cancers [26]. This situation actually calls for the development of inhibitors, which block both EGFR and Her-2. GW 2016 is such an inhibitor; it blocks both receptors with an IC_{50} of 12 nM [27]. It is likely that more pan-Her kinase inhibitors will be developed and enter the clinic.

6. Antibodies to EGFR

Anti EGFR antibodies are currently in clinical trials and in due course their therapeutic utility will be established. Two monoclonal antibodies, Erbitux [16] and ABX-EGF [14,15] are currently in clinical trials. In principle one would expect antibodies to be effective only if they down-regulate the receptor and lead to its degradation. This is actually equivalent to the sustained blockade of the EGFR by an irreversible kinase inhibitor. Time will tell whether these antibodies can be clinically effective. Since a significant fraction of lung cancers also express Her-2 (25), the utilization of Herceptin is also being examined. In breast cancer Herceptin has been successful in a small fractions of patients, probably in those where Her-2 is a survival-signaling element.

7. Imaging of EGFR and Her-2

In order to make a decision whether to treat with an EGFR kinase inhibitor or another agent targeting EGFR, it is essential to establish that the receptor is over-expressed in the tumor. In order to establish the presence of EGFR in the tumor we have recently developed ^{11}C and ^{18}F labeled EGFR kinase inhibitors, for imaging tumors in vivo. Using a reversible ^{18}F-EGFR kinase inhibitor [28] we showed that the agent can image an EGFR over-expressing tumor in a similar fashion to ^{18}FDG, confirming the utility of such inhibitors. Using an ^{11}C EGFR irreversible EGFR kinase inhibitor [29] we can now show that the time window for imaging is much longer since the labeled inhibitor remains for a prolonged period of time at the receptor site, allowing all the non-specific labeling to wash off. Thus imaging of the EGFR, using an irreversible kinase inhibitor, will hopefully become a useful tool to establish the presence of the EGFR. This will allow the better selection of patients for the treatment with anti-EGFR agents. New imaging agents, which target Her-2 and of both EGFR and Her-2 are being developed in order to cover a wider ranges of lung cancers in which her-2 or both receptors are over-expressed.

8. Will the targeting of the Her family be sufficient?

The overexpression of EGFR and its family members is the hallmark of numerous cancers and also of a significant portion of non-small cell lung cancer (NSCLC). It remains unclear at this point whether when EGFR is overexpressed an anti-EGFR therapy will be effective. Studies summarized in Artega [30] show that such a correlation was found in some studies for some cancers but was not found in a significant number of studies. Thus it remains unclear at this point, how to assess the likelihood of success of an anti-EGFR therapy and actually how exactly to choose the patients with lung cancer for the treatment with anti-EGFR therapy. So far we discussed the outcome of the blockade of the EGFR family and its possible therapeutic consequence. One may consider another strategy: using the EGFR in the over-expressing cell as the Trojan horse in order to have a molecule, which induces apoptosis of the cell, enter the cell. Cellular studies performed recently in our laboratory show that PEGylated liposomes, which carry EGF and are bound with pro-apoptotic molecules induce the rapid demise by apoptosis of glioma cells, which over-express the EGFR with no ill effects on cells with normal levels of the EGFR (Shir and Levitzki, in preparation). This strategy is currently being investigated in animal models.

9. Combinatorial signal transduction therapy

Once other signaling elements, which drive the various forms of lung cancer, are identified, one has the (currently theoretical) possibility of combining a few signal transduction inhibitors to achieve better efficacy. Since in a significant fraction of lung cancers K-ras is mutated, an inhibitor of the Ras pathways can be added to the anti-EGFR agent(s) in such instances. Another candidate is the PI-3-kinase–PDK1–PKB/Akt pathway. Since Akt is persistently activated in lung cancers, it seems that this anti-apoptotic pathway, plays an important role in lung cancer [31,32]. Furthermore, the silencing or deletion of PTEN, the negative regulator of this pathway, seems also to occur in an increasing number of tumor specimens [33]. Thus targeting the PKB/Akt pathway, in conjunction with the targeting of EGFR may be an important strategy to consider. It is likely that once the signaling network of the various forms of lung cancer will be deciphered, the other elements, for which signaling needs to be inhibited, will be discovered. Then, one may try to combine a number of signal transduction inhibitors in order to sensitize more effectively the cancer cell to pro-apoptotic stimulation. We have adopted such an approach in our attempts to inhibit the growth of the metastatic prostate cancer cell (Ben-Bassat and Levitzki, unpublished experiments).

10. Combining EGFR directed agents with other therapies

Clinical trials have recently shown that the combination of Iressa with CDDP is not effective [5,34]. This was actually surprising since pre-clinical experiments suggested that such a combination was effective [35]. However, one must always remember that the biology of xenografts is different from actual human tumors. The Iressa/CDDP trials probably suggest that EGFR is not essential for the survival of the tumor cell. Had EGFR been a survival element, its blockade would have resulted in its sensitization to CDDP, as was demonstrated in pre-clinical studies. The fact that Iressa's action was not even additives with CDDP suggests that both act on a common target. This puzzle still awaits more experimentation and a better understanding of the tumor cell signaling network in lung cancers.

11. Targeting signaling elements in the EGFR signaling pathway

One of the questions to be tackled is whether targeting the EGFR pathway is best achieved by blocking the receptor itself or by blocking some of its downstream elements. Fig. 2 examines the network of EGFR signaling. One of the major pathways of EGFR is the Raf–Ras–Mek–Erk. Since K-ras is mutated in a large fraction of lung cancers [36], it is not surprising that Iressa has less of an effect on these patients. The question remains whether Iressa can still be effective when used in combination with a Ras signaling inhibitor. This may involve a Ras inhibitor, a Raf kinase inhibitor [37] or a Mek inhibitor [38]. This is actually a testable hypothesis since EGFR signaling bifurcates to other downstream signaling elements, which contribute to the oncogenic phenotype of the cancer cell (Fig. 2). For example, it has been reported that PKB/Akt is persistently active in lung cancer [31,32]. This highly important anti-apoptotic enzyme may indeed be a very significant oncogenic element in

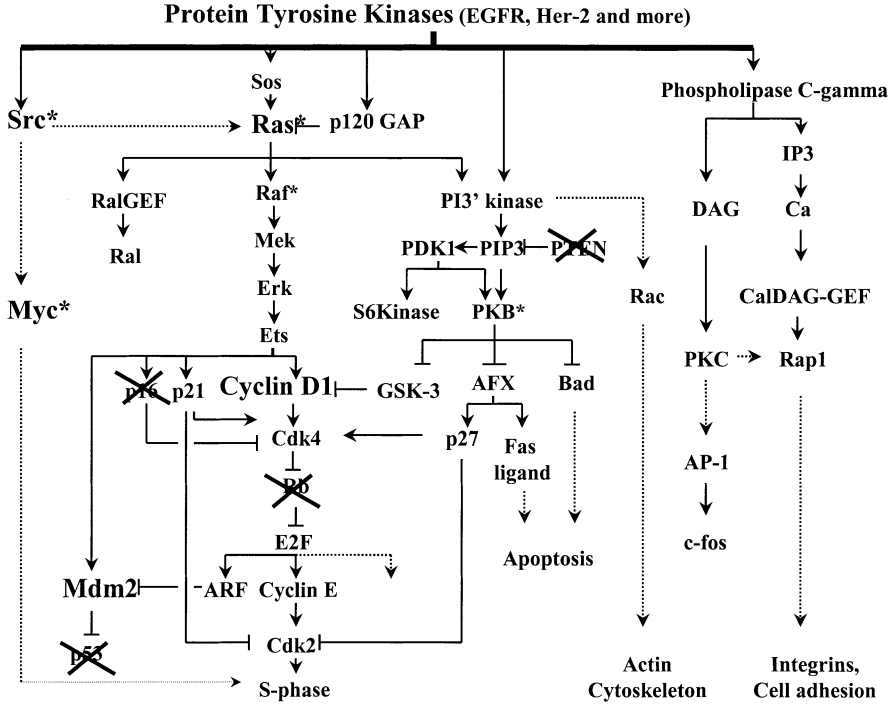

Fig. 2 The signaling network of EGFR. Protein tyrosine kinases, like the EGFR, transduce signals to a number of signal transduction modules as depicted. Thus if certain elements in the downstream signaling complexes are mutated such that their signaling is no more dependent on EGFR, it is still worthwhile to inhibit EGFR, since it still activates other elements in its signaling network. It will probably be beneficial to combine the EGFR signaling inhibitor with signaling inhibitors aimed at the mutated element as well as with other signaling inhibitors aimed at elements which are down stream to EGFR and other receptor tyrosine kinases such as her-2 and PDGFR (see text). For example, if PTEN is attenuated or deleted, the inhibition of PKB along with the inhibition of EGFR may be useful. This is especially true for PKB, since its activation also emanates from other up-stream elements. Elements depicted with a star indicate that antagonists are available.

lung cancers. Since its activation is not only EGFR dependent but is downstream to many growth factor receptors and G protein coupled mitogenic receptor pathways, it may be useful to combine a PKB/Akt inhibitor [39] with Iressa or an anti-EGFR antibody. We adopted the approach of combinatorial signal transduction therapy in order to inhibit the metastatic prostate cancer cell, which is driven by PKB, Jak2 and the EGFR receptor family (mainly EGFR and Her-2).

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