

Growth factor pathway switching: implications for the use of gefitinib and trastuzumab

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Abstract Over-expression or aberrant signalling of the erbB family members epidermal growth factor receptor (EGFR) and HER2 (erbB2/neu) have been associated with the pathogenesis of the malignant phenotype. In addition, high levels of EGFR and HER2 expression have been shown to correlate with poor prognosis and also implicated in disease progression. Signal transduction inhibitors (STIs) have been developed with specifically target these receptors and include the small molecule tyrosine kinase inhibitor gefitinib (Iressa™) which targets the EGFR and the humanised monoclonal antibody trastuzumab (Herceptin™), which has anti-tumour activity against HER2. Studies however, have indicated that de novo or acquired resistance to these agents is a major clinical problem. Cancer cells are highly adaptive and can readily switch from one receptor signalling pathway to another in order to maintain growth or cell survival, a process paradoxically, that in many instances is induced by the anti-tumour agents themselves, ultimately limiting their activity and promoting resistance. Evidence is accumulating which demonstrates that signalling interplay occurs between the EGFR/HER2 and the insulin-like growth factor -1 receptor (IGF-1R) and the article will focus on the growth factor pathway switching that occurs between these receptors which can influence the effectiveness gefitinib and trastuzumab.

Keywords: breast cancer; EGFR; gefitinib; HER2; IGF-1R; resistance; trastuzumab

Introduction

The interest of the Tenovus Centre for Cancer Research in targeting the epidermal growth factor receptor (EGFR) has its origins in the early to mid-1980s when it was first recognised that the EGFR, together with its family member HER2 (c-erbB-2), were found at high levels in approximately 25% of breast cancers where their presence often heralded a more aggressive cancer phenotype and consequently poor patient outlook [1,2]. At that time, it was thought that if the over-expression of these receptor tyrosine

kinases was directing unfavourable tumour characteristics, such as increased cell growth and invasiveness, then their subsequent targeting and inactivation might restore a more normal phenotype which would be to the benefit of the breast cancer sufferer. Twenty years later, we are now amassing the pharmacological tools to selectively attack these receptor proteins which include antibodies such as trastuzumab (Herceptin™) and small molecule tyrosine kinase inhibitors such as gefitinib (Iressa™) and it is encouraging that tumour remissions have been observed in breast cancer patients treated with these drugs [3,4].

Disappointingly, however, the recent clinical experience across a range of cancer types including breast cancer is revealing that despite anti-EGFR and anti-HER2 agents demonstrating some anti-tumour activity, there is a high level of de novo resistance to such treatments [5–10], where over-expression of the EGFR and HER2 is clearly not the sole determinant of

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response to anti-erbB therapies [3,10–12], and moreover, even in responders, the acquisition of resistance and hence disease relapse, occurs fairly rapidly [6–10]. Such adverse phenomena, which serve to limit the overall therapeutic impact of these new agents, implies the existence of a greater complexity involved in EGFR/HER2 signalling network than was previously assumed. Indeed, evidence is accumulating which demonstrates that signalling interplay occurs between the EGFR/HER2 and other growth factor receptors. In this light, the article will focus on the emerging concept of growth factor pathway switching as a means of influencing the effectiveness of anti-growth factor regimes, paying particular attention to the interplay between EGFR/HER2 and the insulin-like growth factor -1 receptor (IGF-1R) where our laboratory observations clearly demonstrate that altered IGF-1R signalling can influence the effectiveness of gefitinib and trastuzumab.

Growth factor pathway switching

Cancer cells are generally regarded as having a high level of plasticity with respect to their use of growth regulatory mechanisms. Stemming from this, it is increasingly evident that despite tumour cells having adopted a dominant growth pathway as a result of the aberrant expression of a particular class of growth factor receptor, they are not necessarily incapable of responding to other paracrine, endocrine or even autocrine growth factors that may be present. Conceptually, therefore, an ability to switch to using these alternative growth signalling pathways during drug treatment represents an obvious means of limiting the actions of highly targeted therapies. As proof of principal of this concept, the substantial anti-tumour response to gefitinib demonstrated by our EGFR positive tamoxifen resistant breast cancer cells, is easily reversed by several non-EGF like growth factors that reactivate cell proliferation and survival signalling, including heregulins and IGFs [13,14]. Thus, although gefitinib is able to efficiently reduce EGFR signalling onto MAPK and AKT in these cells to inhibit their growth [15,16], IGFs acting through the IGF-1R readily reactivate these signalling molecules and thereby re-establish tumour cell growth [14]. Clearly, an *in vivo* cellular environment rich in non-EGF like growth factors which have the capacity to impinge on growth-related pathways, would be expected to protect cancer cells against agents which specifically target the EGFR (and HER2) and compromise their anti-tumour properties.

Significantly, the phenomenon of growth factor pathway switching is not just a passive process dependent upon the availability of alternative growth factors arising in a paracrine or autocrine manner. We

have recently demonstrated that it contains a strong adaptive component that may be directly induced by the selective inhibition of a previously aberrant growth factor pathway. In our experience this often involves increased signalling through the IGF-1R which appears a common mechanism of promoting resistance to either EGFR or HER2 blockade [17]. For example, our *in vitro* models of acquired gefitinib resistance in breast cancer have shown that despite showing good initial growth inhibitory responses to gefitinib, chronic exposure of both our tamoxifen and fulvestrant (Faslodex™) resistant cell lines to the inhibitor resulted in the eventual emergence of gefitinib resistant variants which showed up-regulation of multiple IGF-1R signalling components and increased sensitivity to IGF-1R inhibition with respect to growth [17,18]. Indeed, the involvement of the IGF-1R signalling in mediating the acquired gefitinib resistant phenotype has also been shown in other model systems generated from prostate and lung cancer cells [18,19] which highlights its importance across multiple cancer types. Interestingly, although IGF-1R signalling plays a role in the maintenance of growth in the established acquired resistant phenotype, its ability to prevent apoptosis and promote survival [20] may also be critical in limiting the effectiveness of anti-EGFR/HER2 strategies in the early phase of use of the inhibitors. This appears to stem from the ability of EGFR/HER2 to suppress signalling from other growth factor receptors such as the IGF-1R, where the subsequent blockade of EGFR/HER2 would relieve the inhibition on the suppressed pathway to efficiently promote its signalling and instigate tumour cell growth. As such, we have shown that although total IGF-1R expression remains unaltered, exposure to gefitinib can induce a marked increase in the activity of the IGF-1R in A549 lung cancer cells which demonstrate partial sensitivity (approximately 40% growth inhibition) to the inhibitor [19]. Mechanistically, this may originate from the ability of EGFR to sequester IRS-1, a classically described adaptor protein for IGF-1R as we have shown that although challenge of our tamoxifen resistant breast cells with gefitinib immediately results in a decrease in activated IRS-1 at the 896 tyrosine residue [21] which is known to bind Grb-2, leading to MAPK signalling [22], it never the less simultaneously promotes IRS-1 activity at the 612 tyrosine residue [21] which is known to result in the formation of a pYMXM consensus motif for PI3-kinase binding and subsequent activation of AKT [22], a well-established potent survival signal [23]. Indeed, evidence is accumulating which indicates that an association exists between elevated IGF-1R expression/signalling and its downstream components such as AKT and resistance to drugs which inhibit erb family signal transduction in various cancers. For example,

IGF-1R via PI3-kinase/AKT activation has been shown to mediate resistance to the selective EGFR tyrosine kinase inhibitor AG1478 in glioblastoma cells [24] and also the anti-EGFR monoclonal antibody 225 in DiFi human colorectal cancer cell line [25].

Together with the increased dependency on IGF-1R signalling, we have observed that the acquisition of resistance to gefitinib also promotes cross-resistance to trastuzumab. Thus although we have shown that our HER2 positive tamoxifen resistant breast cancer cells are substantially growth inhibited by this anti-HER2 agent, sensitivity to trastuzumab is lost in our gefitinib resistant variant [17]. Paradoxically, this occurs despite the fact that they have elevated levels of HER2 expression and activity [17]. Importantly, IGF-1R signalling has also been implicated in modulating responses to trastuzumab [26] and it has also been observed that IGF-1R can uni-directionally activate HER2, which involves a physical association of the two receptors [27]. Similarly, our gefitinib resistant breast cancer cells also show evidence of the existence of a physical interaction between the IGF-1R and HER2, which furthermore, co-localise at the tumour cell membranes [17]. Interestingly, we have also observed that vice versa, the acquisition of resistance to trastuzumab is accompanied by a cross-resistance to gefitinib and moreover, the trastuzumab resistant breast cancer cells also show an up-regulation of components of IGF-1R signalling [28]. Such data further adds to the observation that the IGF-1R is increasingly becoming a key therapeutic target to potentially improve the efficacy of erBB inhibitors.

Growth factor pathway switching can occur not only as a result of changes such as those seen during the development of acquired resistance, but can also occur very rapidly and may modulate initial sensitivity to EGFR inhibitory agents. Thus, together with the facilitation of IGF-1R activity, following blockade of EGFR signalling [19], we have recently identified an important reverse element to this pathway switching that involves a capacity of the IGF-1R to transphosphorylate the EGFR on specific tyrosine residues to further contribute to gefitinib resistant growth, with this reverse signalling mechanism again being evident in the partially responsive A549 cells and which contain high IGF-1R signalling [19]. Pre-existence of IGF-1R signalling, or its rapid induction by anti-EGFR strategies, may thus act to efficiently limit the anti-tumour activity of gefitinib and most likely, trastuzumab.

Future therapeutic implications stemming from growth factor pathway switching

An appreciation of the phenomenon of growth factor pathway switching may in the future, allow us to

devise strategies to improve the anti-tumour properties of drugs such as gefitinib and trastuzumab. As such, we envisage that the targeting of these additional growth factor pathways may extend the efficacy of anti-EGFR and anti-HER2 strategies and possibly even create responses in previously unresponsive cells. Certainly, we have already shown that the acquisition of gefitinib resistance in our tamoxifen resistant breast cancer cells can be delayed or even prevented by the combination of gefitinib plus an IGF-1R inhibitor, confirming that IGF-1R is a key compensatory cell survival mechanism acquired during gefitinib resistance [29]. Indeed, it has also been shown that growth inhibition and the apoptosis-inducing effects of gefitinib can be enhanced by the co-inhibition of IGF-1R signalling in a range of breast cancer cell lines [30]. Co-targeting the EGFR and the IGF-1R has also been effective in synergistically sensitising glioma cells to apoptosis [31] and recently, the blockade of IGF-1R signalling with the humanised anti-IGF-1R antibody h7C10 was shown to enhance the anti-tumour effects of vinorelbine and the anti-EGFR antibody 225 in human breast and lung cancer xenografts [32]. It is interesting to note that a bispecific antibody, known as Di-diabody, that targets both the IGF-1R and EGFR has been developed and has been shown to possess *in vivo* anti-tumour effects in colorectal and pancreatic carcinoma xenografts [33].

In conclusion, a body of preclinical evidence is accumulating which implicates the signalling interplay between the EGFR and the IGF-1R as playing an important role in mediating responses to anti-EGFR/HER2 strategies. It is our hope that the identification of key players involved in growth factor pathway switching will ultimately lead to the more productive use of anti-growth factor agents in the treatment of breast cancer and the lessons learnt in this important disease will find general applicability in other prevalent cancer types.

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References

1. Gee JMW, Robertson JF, Gutteridge E, *et al.* Epidermal growth factor receptor/HER2/insulin-like growth factor

- receptor signalling and oestrogen receptor activity in clinical breast cancer. *Endocr Relat Cancer* 2005; **12**(Suppl 1): S99–S111.
2. Nicholson RI, McClelland RA, Finlay P, et al. Relationship between EGFR, c-erbB-2 protein expression and Ki67 immunostaining in breast cancer and hormone sensitivity. *Eur J Cancer* 1993; **29A** (7): 1018–1023.
 3. Yeon CH, Pegram MD. Anti-erbB-2 antibody trastuzumab in the treatment of HER2-amplified breast cancer. *Invest New Drugs* 2005; **23**(5): 391–409.
 4. Agrawal A, Gutteridge E, Gee JM, Nicholson RI, Robertson JF. Overview of tyrosine kinase inhibitors in clinical breast cancer. *Endocr Relat Cancer* 2005; **12**(Suppl 1): S135–S144.
 5. Gee JMW, Gutteridge E, Robertson JF, Wakeling AE, Jones HE, Nicholson RI. Biological markers during early treatment of tamoxifen-resistant breast cancer with gefitinib ('Iressa'). *Breast Cancer Res Treat* 2004; **88**(Suppl 1): S32 (Abstract 307).
 6. Kelly K, Averbuch S. Gefitinib: phase II and III results in advanced non-small cell lung cancer. *Semin Oncol* 2004; **31**(Suppl 1): 93–99.
 7. Rothenberg ML, Lafleur B, Washington MK, et al. Changes in epidermal growth factor receptor signalling in serum and tumour biopsies obtained from patients with progressive metastatic colorectal cancer (MCRC) treated with gefitinib (ZD1839): An Eastern Cooperative Oncology Group Meeting. *Proc Am Soc Clin Oncol* 2004; **21**: 59 (Abstract 3000).
 8. Ranson M, Hammond L, Ferry D, et al. Kris ZD1839 a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid malignant tumours: results of phase I trial. *J Clin Oncol* 2002; **20**: 2240–2250.
 9. Schiller JH. New directions for ZD1839 in the treatment of solid tumours. *Semin Oncol* 2002; **30**(1 Suppl 1): 49–55.
 10. Ocana A, Cruz JJ, Pandiella A. Trastuzumab and antiestrogen therapy: focus on mechanisms of action and resistance. *Am J Clin Oncol* 2006; **29**(1): 90–95.
 11. Arteaga CL. Epidermal growth factor receptor dependence in human tumours: more than just expression. *Oncologist* 2002; **7**(Suppl 4): 30–39.
 12. Saltz LB, Meroplo NJ, Loehrer PJ, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor. *J Clin Oncol* 2004; **22**: 1201–1208.
 13. Hutcheson IR, Knowlden JM, Barrow D, et al. Heregulin-induced AKT activation promotes growth of gefitinib ('Iressa', ZD1839)-treated, tamoxifen-resistant breast cancer cells. *Breast Cancer Res Treat* 2003; **82**(Suppl 1): S170 (Abstract 1008).
 14. Knowlden JM, Hutcheson IR, Barrow D, Gee JM, Nicholson RI. IGF-1R signalling in tamoxifen-resistant breast cancer: a supporting role to the EGFR. *Endocrinology* 2005; **146**(11): 4609–4618.
 15. Knowlden J, Hutcheson IR, Jones HE, et al. Elevated levels of EGFR/c-erbB2 heterodimers mediate an autocrine growth regulatory pathway in Tamoxifen resistant MCF-7 cells. *Endocrinology* 2003; **144**(3): 1032–1044.
 16. Jordan NJ, Gee JMW, Barrow D, Wakeling AE, Nicholson RI. Increased constitutive activity of PEBB/Akt in tamoxifen resistant breast cancer MCF-7 cells. *Breast Cancer Res Treat* 2004; **87**(2): 167–180.
 17. Jones HE, Gee JMW, Taylor KM, et al. Development of strategies for the use of anti-growth factor treatments. *Endocr Relat Cancer* 2005; **12**(Suppl 1): S173–S182.
 18. Jones HE, Goddard L, Gee JMW, et al. Insulin-like growth factor-1 receptor signalling and acquired resistance to gefitinib (ZD1839; Iressa) in human breast and prostate cancer cells. *Endocr Relat Cancer* 2004; **11**: 1–22.
 19. Jones HE, Gee JMW, Barrow D, Holloway B, Tonge D, Nicholson RI. Maintenance of EGFR phosphorylation by the IGF-1R in the presence of gefitinib in lung cancer cells: co-targeting the EGFR and IGF-1R maximises anti-tumour effects. *Proceedings of the 4th International Symposium on Signal Transduction Modulators in Cancer Therapy* 2006, Amsterdam, The Netherlands (Abstract P.210).
 20. Adams TE, Epa VC, Garrett TPJ, Ward CW. Structure and function of the type 1 insulin-like growth factor receptor. *Cell Mol Life Sci*. 2000; **57**: 1050–1093.
 21. Knowlden JM, Hutcheson IR, Gee JM, Barrow D, Wakeling AE, Nicholson RI. Gefitinib (Iressa) promotes IGF-1R signalling in tamoxifen resistant MCF-7 breast cancer cells: role of IRS-1. *Breast Cancer Res Treat* 2004; **88**(Suppl 1): S145 (Abstract 3097). *27th Annual San Antonio Breast Cancer Symposium*, San Antonio, TX, USA.
 22. Hers I, Bell CJ, Poole AW, et al. Reciprocal feedback regulation of insulin receptor and insulin receptor substrate tyrosine phosphorylation by phosphoinositide 3-kinase in primary adipocytes. *Biochem J* 2002; **368**: 875–884.
 23. Cheng JQ, Lindsley CW, Cheng GZ, Yang H, Nicosia SV. The Akt/PKB pathway: molecular target for cancer drug discovery. *Oncogene* 2005; **24**(50): 7482–7492.
 24. Chakravati A, Loeffler JS, Dyson NJ. Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signalling. *Cancer Res* 2002; **62**: 200–207.
 25. Liu B, Fang M, Lu Y, Mendelsohn J, Fan Z. Fibroblast growth factor and insulin-like growth factor differentially modulate the apoptosis and G1 arrest induced by anti-epidermal growth factor receptor monoclonal antibody. *Oncogene* 2001; **20**: 1913–1922.
 26. Lu Y, Zi X, Zhao Y, Mascarenhas D, Pollak M. Insulin-like growth factor-1 receptor signaling and resistance to Trastuzumab (Herceptin). *J Natl Cancer Inst* 2001; **93**(24): 1852–1857.
 27. Balana ME, Labriola L, Salatino M, et al. Activation of ErbB-2 via a hierarchical interaction between ErbB-2 and type I insulin-like growth factor receptor in mammary tumour cells. *Oncogene* 2001; **20**(1): 34–47.
 28. Hutcheson IR, Gee JMW, Barrow D, Jones HE, Wakeling AE, Nicholson RI. Treatment of tamoxifen resistant MCF-7 breast cancer cells with either gefitinib (Iressa) or trastuzumab (Herceptin) generates cross-resistant phenotypes. *Breast Cancer Res Treat* 2004; **88**(Suppl 1): S145 (Abstract 3097).
 29. Nicholson RI, Hutcheson IR, Knowlden JM, et al. Nonendocrine pathways and endocrine resistance: observations with antiestrogens and signal transduction inhibitors in combination. *Clin Cancer Res* 2004; **10**(1 Pt 2): 346S–352S.
 30. Camirand A, Zakikhani M, Young F, Pollak M. Inhibition of insulin-like growth factor-1 receptor signalling enhances growth-inhibitory and proapoptotic effects of gefitinib

- (Iressa) in human breast cancer cells. *Breast Cancer Res Treat* 2005; **7**(4): R570–R579.
31. Steinbach JP, Eisenmann C, Klumpp A, Weller M. Co-inhibition of epidermal growth factor receptor and type 1 insulin-like growth factor receptor synergistically sensitizes human malignant glioma cells to CD95L-induced apoptosis. *Biochem Biophys Res Comm* 2004; **321**(3): 524–530.
 32. Goetsch A, Gonzalez A, Leger O, *et al.* Recombinant humanized anti-insulin-like growth factor receptor type I antibody (h7C10) enhances the antitumor activity of vinorelbine and anti-epidermal growth factor receptor therapy against human cancer xenografts. *Int. J Cancer* 2005; **113**(2): 316–328.
 33. Lu D, Zhang H, Koo H, *et al.* A fully human recombinant IgG-like bispecific antibody to both the epidermal growth factor receptor and the insulin-like growth factor receptor for enhanced antitumor activity. *J Biol Chem* 2005; **280**(20): 19665–19672.