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Activation of alternative HER receptors mediates resistance to EGFR tyrosine kinase inhibitors in breast cancer cells

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The response rate to EGFR inhibitors may be poor and unpredictable in cancer patients with EGFR expression itself being an inadequate response indicator. There is limited understanding of the mechanisms underlying this resistance. Here we have provided a molecular mechanism of alternative HER receptor activation (ErbB receptor family members) in mediating resistance to EGFR TKIs in breast cancer cells. Using both Förster Resonance Energy Transfer (FRET) which monitors in situ HER receptor phosphorylation as well as classical biochemical analysis, we have shown that the specific tyrosine kinase inhibitors (TKIs) of EGFR (HER1), AG1478 and Iressa (Gefitinib) decreased EGFR and HER3 phosphorylation through the inhibition of EGFR/HER3 dimerization. Consequent to this, we demonstrate that cleavage of HER4 and dimerization of HER4/HER2 occur together with reactivation of HER3 via HER2/HER3, leading to persistent HER2 phosphorylation in the now resistant, surviving cells. These drug treatment-induced processes were found to be mediated by the release of ligands including heregulin and betacellulin that activate HER3 and HER4 via HER2. Whereas an anti-betacellulin antibody in combination with Iressa increased the anti-proliferative effect in resistant cells, ligands such as heregulin and betacellulin rendered sensitive SKBR3 cells resistant to Iressa. These results demonstrate the role of drug-induced autocrine events leading to the activation of alternative HER receptors in mediating resistance to EGFR tyrosine kinase inhibitors (TKIs) in breast cancer cells, and hence specify treatment opportunities to overcome resistance in patients.

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Combined targeting of DNA repair and AKT survival pathways enhance temozolomide therapeutic activity in melanoma

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Background: Melanoma, the most fatal skin cancer, has increased in incidence by 15-fold in the past 40 years, the fastest rate increase of any human malignancy. This disease metastasizes rapidly and is highly resistant to chemotherapy and other treatments. At this time, a new oral alkylating agent, Temozolomide (TMZ) is an effective drug with modest therapeutic activity for the treatment of melanoma, however, intrinsic and acquire resistance is the major cause of the reduction in effectiveness and in improving overall survival by this single agent. Although many combination treatments using immunotherapy, chemotherapy, and bio-chemotherapy have been tested in clinical studies, nearly all trials of combination therapies have failed. Thus, it is imperative to develop more targeted approaches to augment substantial benefit of treatment.
Methods: In this study, using melanoma cell lines we examined therapeutic activity of targeting based combinations: (i) combining methoxyamine (MX), an inhibitor of base excision repair (BER), with TMZ to block the repair of DNA adducts that are account for about 80% DNA lesions produced by TMZ, (ii) combining API-2, a small-molecule Akt inhibitor, with TMZ to inhibit TMZ-induced activation of AKT pathway, an important molecular event implicated in tumor cell survival and chemo resistance. We hypothesized that the combination of TMZ with MX and API-2 would synergistically enhance anti-tumor effect of TMZ through targeting two major resistant factors: DNA repair and AKT mediated anti-apoptosis.
Results: MX enhanced TMZ cytotoxicity in A375, WM9 and WM164 melanoma cells in vitro and in xenografts setting. The potentiation of TMZ by MX was through its activity to specifically bind to an abasic site, which turns the repairable DNA damage into a lethal lesion, leading to DNA strand breaks and apoptosis. However, we found that AKT was activated in response to either TMZ alone or in combination with MX, showing the induction of phosphorylated AKT. Thus, the combining with API-2 (1 microM) efficiently inhibited AKT activation and significantly increased apoptosis, 4 to 5-fold higher than TMZ alone. The increased apoptotic death was mediated by Bax-activation. Similarly, siRNA-mediated reduction of AKT expression sensitized melanoma cells to TMZ-cytotoxicity.
Conclusion: These results strongly support the hypothesis that clinical benefit could be obtained by combining TMZ with blocker of DNA repair and inhibitor of the AKT pathway.

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An inducible expression system to study the EGFR-T790M gefitinib-resistance mutation in a human lung cancer cell line

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Background: Lung cancer patients whose tumours harbour somatic mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) respond to the reversible EGFR inhibitors gefitinib and erlotinib. One such mutation is the deletion of aminoacids 746–750, hereafter referred to as EGFR-Del. Resistance to gefitinib or erlotinib invariably develops and is often mediated by the appearance of the T790M mutation in EGFR. The T790M mutant is believed to be sensitive to irreversible EGFR inhibitors such as CL-387,785. To study the impact of EGFR-T790M expression in a relevant cell line, doxycycline (DOX)-inducible expression of EGFR-Del/T790M was established in the human lung cancer cell line HCC827, which expresses EGFR-Del and is very sensitive to gefitinib.
Materials and Methods: HCC827 cells capable of DOX-inducible expression of FLAG-tagged EGFR-Del (control) or EGFR-Del/T790M were produced by sequential transfection and selection with two different plasmids (Nat. Protoc. 1:803, 2006). Polyclonal pools of selected cells were cloned by plating at low density on 10-cm dishes. Clones expressing FLAG-tagged EGFR-Del ("D") or EGFR-Del/T790M ("T") in the presence of DOX were analysed for drug sensitivity using the MTT assay. Also, the effect of EGFR inhibitors on the activity of EGFR and downstream signalling molecules (Akt and Erk1/2) was analysed by western blotting.
Results: growth of T clones in the presence of DOX decreased the sensitivity of HCC827 cells to gefitinib more than 25 fold (figure 1). This was not observed in control D clones. Also, gefitinib was incapable of inhibiting the activation of EGFR, Akt and Erk1/2 in T clones grown in the presence of DOX. The sensitivity to CL-387,783 was also reduced when T clones were grown in the presence of DOX. However, D and T clones remained equally sensitive to the PI3 kinase (PI3K) inhibitor PI-103, irrespective of DOX treatment.
Conclusions: we have established and validated a robust system for inducible EGFR-T790M-mediated resistance to gefitinib in a relevant cell line. This is the first inducible expression system described for the T790M mutation in an established cancer cell line. Expression of EGFR-T790M also decreased the sensitivity of cells to an irreversible EGFR inhibitor. Our results suggest that targeting downstream signalling molecules (such as PI3K) might be a better strategy for overcoming T790M-mediated resistance in the clinic when compared to irreversible EGFR inhibition.

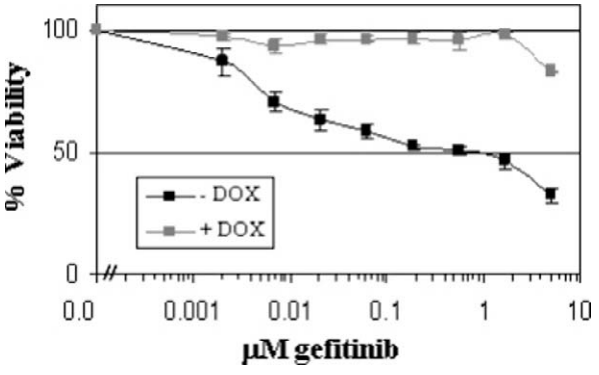

Figure 1. MTT assay – T clone.

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MDR-transporters, namely Pgp, MRP1 and vault protein LRP, as poor predictive markers of tamoxifen efficiency in estrogen receptor positive breast cancer tumours

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Background: Estrogen receptors (ER) are determinants of tamoxifen (Tam) sensitivity of breast cancer but the treatment are not effective in all the patients with ER-positive tumors. We supposed that among the