in a murine renal tumor model (Renca). Despite its lack of effects on Renca cell growth in culture, SSG as a single agent in Balb/c mice induced 61% growth inhibition of Renca tumors coincident with increase (2 folds) of tumor-infiltrating macrophages (Mϕ). SSG/IL-2 combination induced 91% growth inhibition of Renca tumors in Balb/c mice accompanied by 4-fold increase in tumor infiltrating Mϕ. IL-2 by itself failed to inhibit Renca tumor growth as reported previously and did not induce tumor-infiltrating Mϕ. The safety of SSG and SSG/IL-2 combination was indicated by the tolerance of the treatments in these mice. Although SSG/IL-2 treatment did not increase tumor-infiltrating T cells in association with Renca tumor growth inhibition in the Balb/c mice, its lack of anti-Renca tumor activity in athymic nude mice indicated a T-cell-dependent immune action mechanism of the combination. Involvement of the tumor-infiltrating Mϕ in the action was supported by the capacity of SSG to increase cytotoxic activity (4 folds) of Raw 264.7 Mϕ against Renca cells in vitro. Evidence of more marked Mϕ increases in spleen in the mice treated with SSG (3 folds) or SSG/IL-2 combination (6 folds) indicated a systemic Mϕ expansion, which recapitulates a prominent feature of murine genetic SHP-1 deficiency and is consistent with in vivo inhibition of SHP-1 by SSG in these mice. These results together demonstrated an anti-Renca tumor activity of SSG that interacts with IL-2 via a T-cell-dependent immune action mechanism in connection with expansion/activation of Mϕ. They provided evidences suggesting a therapeutic potential of SSG in anti-RCC immune therapy and indicating SHP-1as a target molecule for augmenting anti-tumor immunity and improving the efficacy of immune therapies.

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De novo resistance to epidermal growth factor receptor blockade by gefitinib in colorectal cancer cells involves increased insulin receptor isoform A signalling

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Background: Gefitinib (Iressa®) is an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) that has antitumour activity in a range of cancer types. It has become apparent, however, that high EGFR expression and activity do not always predict for response and clinical data have indicated the existence of both *de novo* and acquired resistance to gefitinib in tumours reported to be EGFR positive. In this study, the possible involvement of the insulin receptor isoform A (InsR-A) in *de novo* gefitinib resistance has been investigated in LoVo colorectal human cancer cells.
Materials and Methods: LoVo cells were exposed to 1 μM gefitinib for 7 days. Cell proliferation was determined by evaluating anchorage-dependent growth. Alterations in signalling pathways were assessed by immunocytochemical, Western blotting and/or reverse transcription polymerase chain reaction techniques. Cell sensitivity to the InsR/insulin-like growth factor (IGF)-1 receptor inhibitor ABDP (AstraZeneca) was also measured.
Results: The highly EGFR-positive LoVo cells demonstrated negligible growth inhibition (<15%) after exposure to 1 μM gefitinib. Compared with controls, the gefitinib-treated cells showed a reduction (45%) in phospho-EGFR (Tyr-845) activity, the tyrosine site located in the kinase domain of the receptor. However, levels of activity of the downstream receptor tyrosine sites Tyr-1068 and Tyr-1173 were unchanged in the presence of 1 μM gefitinib, as was the activity of phospho-ERK1/2. Compared with a panel of various cancer cell line types (A549, DU145 and MCF-7), it was noted that the LoVo cells produced markedly higher levels (9-fold) of IGF-II mRNA, unprocessed (~230 kD) inactive pro-IGF-1R protein, mature InsR protein (~130 kD α-subunit) and substantially elevated (10-fold) amounts of InsR-A mRNA, with minimal detection of InsR-B mRNA. Exposure of LoVo cells to insulin and IGF-II resulted in growth promotion (20% and 40%, respectively) and increased phospho-EGFR (Tyr-1068) and EGFR (Tyr-1173) activity over 60 min. Furthermore, challenge with the InsR/IGF-1 receptor inhibitor ABDP resulted in a dose-dependent decrease in basal growth (IC_{50}=0.25 μM). Interestingly, both phospho-InsR and phospho-Akt levels increased (2-fold and 5-fold, respectively) after treatment with gefitinib.
Conclusions: We propose that *de novo* resistance to gefitinib in LoVo colorectal cancer cells may, in part, be directed through the InsR-A, utilising IGF-II as a ligand. The interactions between InsR and EGFR are currently being further assessed. Evidence suggests that InsR-A overexpression is important in the promotion of cancer growth in IGF-II-producing tumours (Sciacca *et al*. Oncogene 2002;21:8240–50). Co-targeting cells with gefitinib and an InsR/IGF-1R inhibitor may prove more effective than gefitinib alone.
Iressa is a registered trademark of the AstraZeneca group of companies

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Velcade, an NF-kb inhibitor, enhances tamoxifen sensitivity in Akt-induced resistant breast cancer xenograft tumors

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Tamoxifen resistance is the underlying cause of treatment failure in a significant number of patients with breast cancer. Only approximately 50% of estrogen receptor α-positive tumors will initially respond to hormonal therapy, and of those tumors that do initially respond, a refractory phenotype will develop in a majority of them within 5 years. Activation of Akt, a downstream mediator in the PI3K signaling pathway has been implicated as one of the mechanisms involved in tamoxifen resistance. Breast cancers with heightened Akt activity are frequently associated with an aggressive disease and resistance to chemo- and hormone-therapy induced apoptosis. In previous studies we have demonstrated that MCF-7 breast cancer cell lines expressing a constitutively active Akt (myrAkt1) are resistant to tamoxifen, both *in vitro* as well as *in vivo* in xenograft models. One potential mechanism is through activation of NF-κB, a known regulator of pro-survival genes. In our model, Akt activity correlated with increased inhibition of IκB (the negative regulator of NF-κB), and increased NF-κB DNA binding. We found that co-treatment with an NF-κB inhibitor or transfection with a non-degradable IκB restored tamoxifen sensitivity to our refractory myrAkt1 MCF-7 cells. Velcade, a specific and reversible inhibitor of the proteasome that prevents NF-κB activation in cancer cells, is currently in clinical development for the treatment of several types of carcinomas, and recently received FDA approval for the treatment of multiple myeloma. We hypothesized that treatment with Velcade would increase the responsiveness of our Akt-induced resistant breast cancer cells to tamoxifen. Nude mice were injected contralaterally with either the myrAkt1 or Control MCF-7 cells, and tumors were allowed to develop to a size of 200 mm^3. Mice were then separated into four (4) treatment groups: Placebo, Velcade as a single agent, tamoxifen as a single agent, or the combination of Velcade with tamoxifen. We found that while Velcade as a single agent had no significant inhibitory effects on our myrAkt1 tumors, tumor growth inhibition was 73% greater with the combination of Velcade and tamoxifen compared to tamoxifen as a single agent. These findings suggest that Velcade as a co-treatment for breast cancer tumors with high levels of Akt activity could be an effective approach for overcoming growth-factor induced resistance and may have implications for future clinical studies of Velcade in breast cancer.

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Computer-aided immunohistochemical analysis of EGFR signaling in paired colorectal cancer and normal colon tissue samples

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Background: The EGFR plays a role in multiple tumor cell processes including proliferation, survival, and susceptibility to chemotherapy. EGFR signaling is complex and multilayered, and appears to have several downstream effector pathways including PI3K-Akt and Ras-Raf-MAPK. The present study aimed to validate and apply a novel computer-aided immunohistochemical (IHC) technique to characterize the status of EGFR signaling in matched colorectal tumor and normal colon tissue samples. We previously presented results in which paired samples were poorly correlated, likely due to differing amounts of confounding stromal tissue in the sample. We present here a re-analysis using a "graphic microdissection" technique in which cell clusters of interest are analyzed separately in an effort to improve correlation between paired samples.
Materials and Methods: Tissue Microarrays (TMA) were made using samples from both cancerous and normal colorectal tissue in 18 patients and stained with antibodies against EGFR, phospho-EGFR (pEGFR), Akt, pAKT, MAPK, and pMAPK. TMA's were quantitatively scored using the Automated Cellular Imaging System (ACIS II, Chromavision, Inc), which couples a computer-controlled brightfield microscope to a camera with imaging analysis software. Prior to image analysis, cell clusters of interest were chosen under the supervision of a gastrointestinal pathologist and circled using the computer mouse. For validation, cell line pellets were analyzed using ACIS II and compared against Western blotting (A431 cells) and ELISA (8 head and neck cancers). Xenograft experiments were also performed in which A431 cells were implanted subcutaneously in nude mice treated with erlotinib versus control vehicle.
Results: ACIS analysis was highly reproducible and results were well correlated with Western blotting and ELISA. The "graphic microdessection" technique of analyzing heterogeneous human samples showed good corre-