Acquired resistance to EGFR tyrosine kinase inhibitors in human head and neck squamous carcinoma cells (HNSCC) and xenografts is associated with an altered metabolic phenotype

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Target audience: Clinicians and researchers working in cancer metabolic imaging and drug development.

Purpose: Acquired resistance to molecular therapeutics is a key challenge in personalized cancer medicine. The identification of mechanisms, as well as early biomarkers of patient relapse, is key for selecting alternative therapies before further disease progression.1 Here we use human head and neck squamous carcinoma cell lines (HNSCC) and xenograft tumours together with magnetic resonance spectroscopy (MRS) to assess the metabolic changes associated with acquired resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), and which could serve as potential non-invasive metabolic imaging biomarkers of drug resistance.

Methods: Acquired resistance to EGFR TKIs was developed in the CAL 27 HNSCC cell line by chronic exposure to gefitinib. The resulting resistant cells (CALR) were approximately 200-fold more resistant to gefitinib, and cross-resistant to erlotinib, lapatinib and afatinib, relative to the control cells (CALS) in growth inhibition assays.2 Metabolite profiles were obtained from CALS and CALR cells cultured in vitro and as subcutaneous xenografts in athymic mice. For this, dual phase extracts were obtained from cells and size-matched tumours (~250mm3) and the aqueous fractions analysed by 1H MRS on a 500MHz Bruker spectrometer. Spectra were processed on MestRe-C v4.9 (University of Santiago de Compostela) and metabolite levels normalized to an internal standard as well as to cell number or tumour sample weight. Multivariate analysis of the 1H MRS data was performed on Simca (Umetrics, v13.0). Protein expression was assessed by western blotting of tumour lysates. Statistical significance was assessed using Student’s t-test with a p≤0.05 considered to be significant.

Results: Whilst acquired resistance to EGFR TKIs had no significant effect on proliferation rate in vitro, CALR xenografts grew significantly faster than CALS in athymic mice. Multivariate analysis of 1H MRS data showed that the CALR and CALS were metabolically distinct. Specifically, CALR tumours exhibited significant increases in levels of glycerophosphocholine (GPC, 2-fold), lactate (1.6-fold), branched chain amino acids (BCAA, 2-fold) and alanine (2.1-fold) as shown in Figure 1A (n=5, p≤0.04). 1H MRS analysis of cells cultured in vitro showed, in agreement with the xenograft tumour data, increases in GPC (2.4-fold), lactate (1.3-fold) and BCAA (1.35-fold) content in CALR compared with CALS cells (n=6, p≤0.03). Western blot analyses of tumour lysates showed increased expression of the glucose transporter GLUT-1 in CALR relative to CALS tumours (Figure 1B).

Discussion: Acquired resistance to TKIs in our HNSCC tumour model is associated with changes in glycolytic, amino acid and choline phospholipid metabolism. The increase in CALR tumour lactate was concomitant with elevated GLUT-1 expression, indicative of increased glycolytic metabolism in the drug resistant tumours.

Conclusions: Our data suggest a potential for metabolic imaging to identify biomarkers of resistance to EGFR TKIs.


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