9 AREG EXPRESSION IS ASSOCIATED WITH RESPONSE TO CETUXIMAB IN A NON-SMALL CELL LUNG CANCER PRIMARY CULTURE

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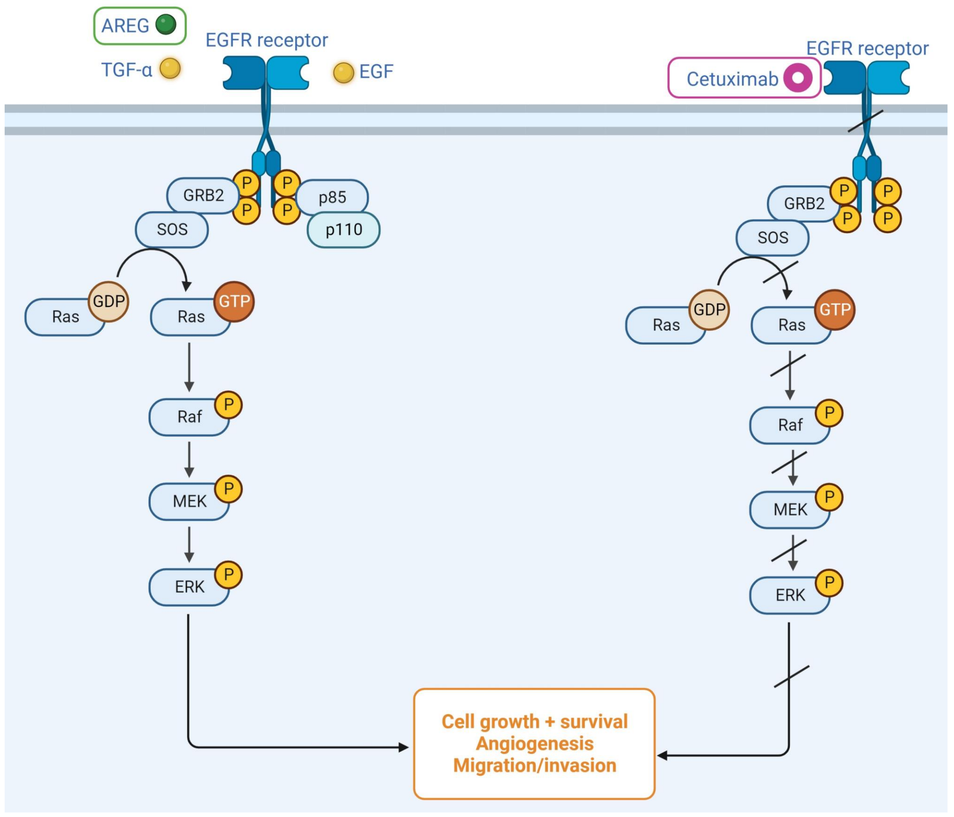
BACKGROUND

Amphiregulin, a transmembrane protein coded by the *AREG* gene, is one of the Epidermal Growth Factor Receptor (EGFR) ligands and induces autophosphorylation of the receptor which, in turn, activates MEK/ERK1/2 and PI3K/AKT pathways. Cetuximab, an anti-EGFR monoclonal antibody, blocks EGFR signaling. Clinical studies in *KRAS* and *BRAF wt* colorectal carcinoma (CRC) patients have shown that high levels of *AREG* in tumor tissue associated with clinical benefit of cetuximab treatment.



OBJECTIVES

- In this study, we aimed to determine the frequency of *AREG* overexpression in a cohort of Non-Small Cell Lung Cancer patients (NSCLC) and the activity of cetuximab in primary cultures (PC) derived from pleural effusions of patients with high *AREG*.

METHODS

Pleural effusions from NSCLC patients were isolated, centrifuged, submitted to erythrocyte removal and cultured in T25 flasks with RPMI and 20% FBS, culture medium was replaced twice per week until cells density was high enough to expand. mRNA levels in PC and FFPE samples of cancer patients were measured using a commercial panel containing 770 mRNA hybridization probes. mRNA expression data from tumor tissues and cell lines was extracted from The Human Protein Atlas database. Cell viability was determined by MTT.

RESULTS

Figure 1: Frequency of *AREG* upregulation in lung cancer cell lines and in lung cancer tissue samples

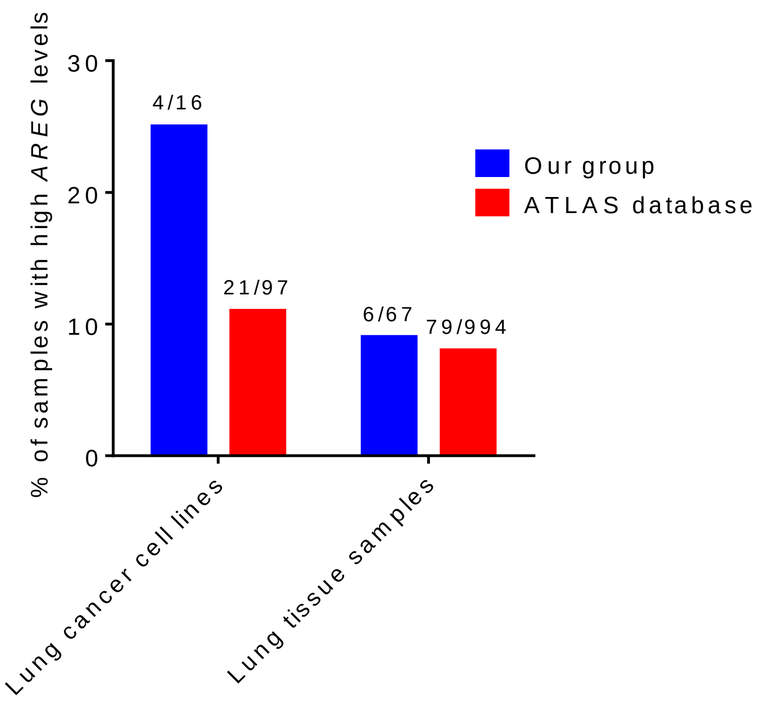


Figure 2: Flow chart of the samples analyzed in the study

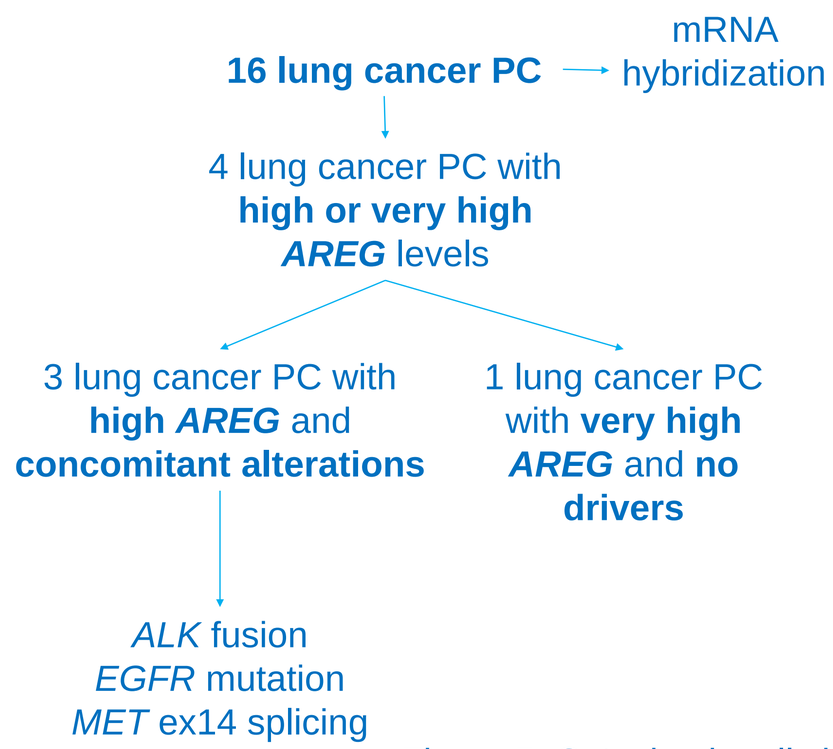


Figure 3: *AREG* levels in the 15 lung cancer PC analyzed

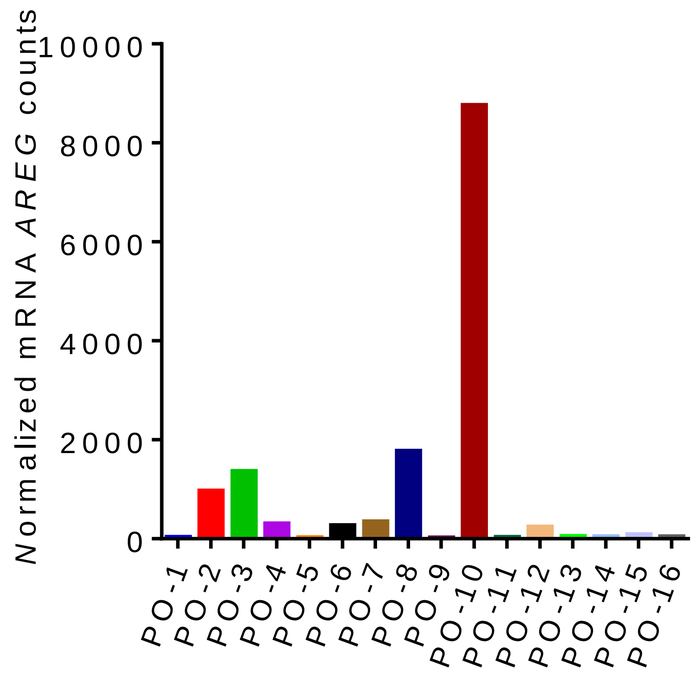
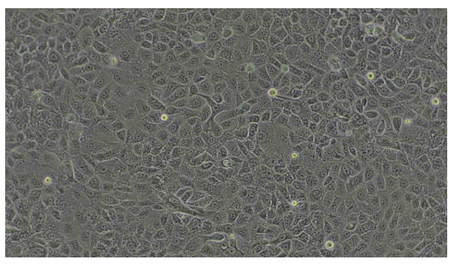


Figure 4: Images of lung cancer PC with high *AREG* levels and an *EML4-ALK* fusion (A), and with very high AREG levels with no more alterations (B)

(A)



(B)

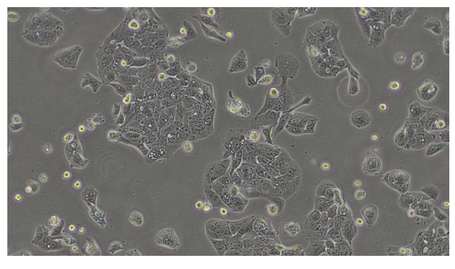
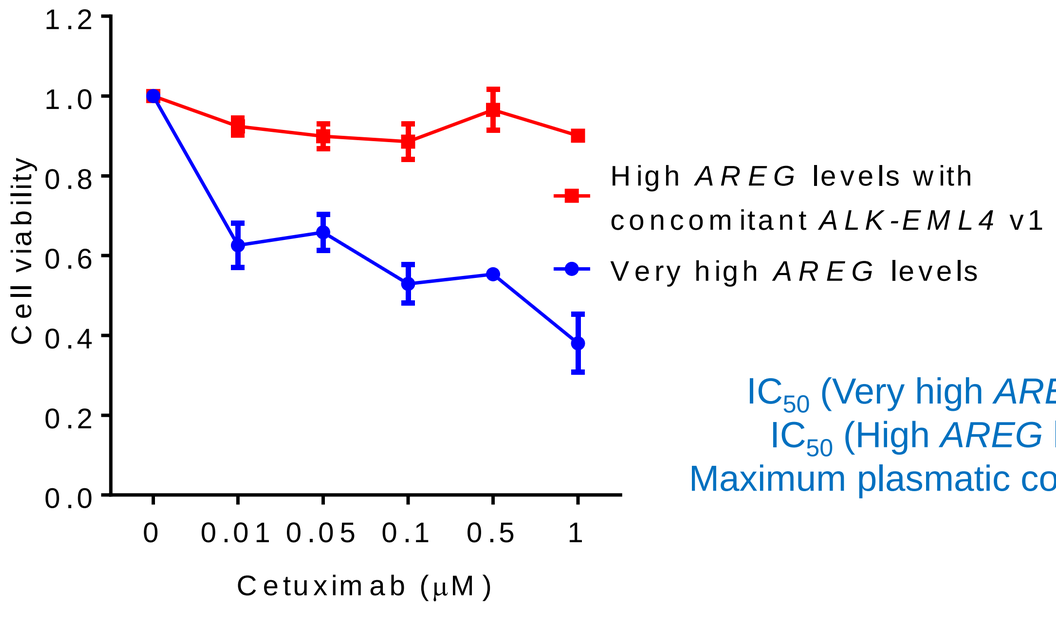


Figure 5: Cetuximab cell viability in cells with very high *AREG* levels and high *AREG* levels with a concomitant *ALK-EML4* fusion



IC_{50} (Very high *AREG* levels) > 1 μM
IC_{50} (High *AREG* levels) = 0.6 μM
Maximum plasmatic concentration = 1.4 μM

CONCLUSIONS

***AREG* mRNA upregulation is found in 8% in NSCLC samples. A primary culture from a patient with very high *AREG* mRNA levels was sensitive to cetuximab. Our results warrant further exploration of *AREG* overexpression as a biomarker of sensitivity to EGFR-targeted antibodies.**