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Short hairpin RNA-directed knockdown of epidermal growth factor receptor in human oesophageal squamous carcinoma cell lines

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Abstract

Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase which activates, upon EGFR binding, a number of signaling pathways including the mitogenic protein kinase pathway (MAPK) and phosphatidylinositol 3-kinase cascade (PI3K). Over expression of EGFR is a common feature in variety of human cancers including lung, colorectal, breast, pancreatic and oesophageal cancers and results in autonomous cell growth, enhanced metastatic potential, tissue invasion and increased resistance to current cancer therapeutics. Thus EGFR has been identified as a potential target in cancer therapeutics. Using the RNA interference (RNAi) pathway, the aim was to specifically knockdown expression levels of endogeneous EGFR in human oesophageal squamous carcinoma cell (HOSCC) lines. The RNAi pathway was initiated through the transfection of three specifically designed short hairpin RNAs (shRNAs) against human EGFR. The shRNAs were specifically designed using bioinformatics tools and their individual knockdown efficacy determined through the introduction of an exogeneous based target reporter systems, psiCHECK and pcieGFP. Expression levels of EGFR were determined using Western blot analysis followed by densitometry. Knockdown of EGFR was achieved by all three EGFR shRNAs in the three HOSCC cell lines (WHCO1, WHCO5 and WHCO6) despite low transfection levels of ~10%. Greastest knockdown of EGFR (85%) was achieved by EGFR sh2 in WHCO5. EGFR sh2 and sh1 resulted in average knockdown of EGFR of ~ 65% in WHCO1 and WHCO5 respectively. Weakest knockdown of EGFR (~ 20%) was obtained by all three EGFR shRNAs following transfection of WHCO6. RNAi-based approaches therefore show substantial potential for the specific and efficient targeting of EGFR in human cancer cells.