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Research Paper

Afatinib radiosensitizes head and neck squamous cell carcinoma cells by targeting cancer stem cells

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ABSTRACT

The dismal prognosis of locally advanced and metastatic squamous cell carcinoma of the head and neck (HNSCC) is primarily due to the development of resistance to chemoradiation therapy (CRT). Deregulation of Epidermal Growth Factor Receptor (EGFR) signaling is involved in HNSCC pathogenesis by regulating cell survival, cancer stem cells (CSCs), and resistance to CRT. Here we investigated the radiosensitizing activity of the pan-EGFR inhibitor afatinib in HNSCC *in vitro* and *in vivo*. Our results showed strong antiproliferative effects of afatinib in HNSCC SCC1 and SCC10B cells, compared to immortalized normal oral epithelial cells MOE1a and MOE1b. Comparative analysis revealed stronger antitumor effects with afatinib than observed with erlotinib. Furthermore, afatinib enhanced *in vitro* radiosensitivity of SCC1 and SCC10B cells by inducing mesenchymal to epithelial transition, G1 cell cycle arrest, and the attenuating ionizing radiation (IR)-induced activation of DNA double strand break repair (DSB) ATM/ATR/CHK2/BRCA1 pathway. Our studies also revealed the effect of afatinib on tumor sphere- and colony-forming capabilities of cancer stem cells (CSCs), and decreased IR-induced CSC population in SCC1 and SCC10B cells. Furthermore, we observed that a combination of afatinib with IR significantly reduced SCC1 xenograft tumors (median weight of 168.25 ± 20.85 mg; $p = 0.05$) compared to afatinib (280.07 ± 20.54 mg) or IR alone (324.91 ± 28.08 mg). Immunohistochemical analysis of SCC1 tumor xenografts demonstrated downregulation of the expression of IR-induced pEGFR1, ALDH1 and upregulation of phosphorylated γH2AX by afatinib. Overall, afatinib reduces tumorigenicity and radiosensitizes HNSCC cells. It holds promise for future clinical development as a novel radiosensitizer by improving CSC eradication.