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Is hypoxia relevant in modulating the effects of the novel antiangiogenic agent, ZD6474, in head and neck cancer?

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Background: Head and neck squamous cell cancer (HNSCC) accounts for approximately 35% of all malignancies worldwide. The majority of HNSCCs are locally advanced and present with poorly vascularized areas within the primary tumor or in metastatic lymph nodes, resulting in regions of hypoxia. Hypoxic tumors are known to be more resistant to both radiation and chemotherapy, which may correlate with increased metastatic potential. Cellular response to hypoxia includes the expression of hypoxia-inducible factor 1α (HIF-1α), a transcription factor that plays a key role in O_2 homeostasis. Synthesis of HIF-1α protein is regulated by activation of the phosphatidylinositol 3-kinase (PI3K) and ERK mitogen-activated protein kinase (MAPK) pathways. These pathways are activated by signaling through receptor tyrosine kinases, such as the binding of transforming growth factor-α to epidermal growth factor receptor (EGFR). ZD6474 is an orally available inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2) tyrosine kinase activity with additional activity against EGFR tyrosine kinase.
Methods: We examined the effect of ZD6474 on downstream signaling of the EGFR pathway leading to HIF-1α synthesis under normoxic (20% O_2) and hypoxic (1% O_2) conditions, using a panel of HNSCC cell lines with varied EGFR expression. We added 0, 0.1, or 1 μM ZD6474 to cell cultures, followed by incubation under normoxic or hypoxic conditions, then harvested supernatants for analysis of VEGF secretion. The cells were used for Western blot analysis at 0, 1, 2, 6, 12, 18, and 24 hours.
Results: ZD6474 inhibited EGFR phosphorylation at two activation sites associated with the MAPK/ERK cascade, Tyr992, and Tyr1068, as well as the activation loop stabilization site Tyr845. The ubiquitination-targeting site on EGFR (Tyr1045) was not affected by ZD6474. ZD6474 was equally effective in inhibiting EGFR activation under normoxic and hypoxic conditions. ERK1/2 and AKT activation were inhibited by ZD6474. Hypoxia-induced HIF-1α synthesis was inhibited by ZD6474 in a dose-dependent manner, although inhibition was transient and varied with cell line. Secretion of VEGF, which is transactivated by HIF-1, also decreased with ZD6474 treatment.
Conclusions: These results suggest that hypoxia is not a limiting factor in the effectiveness of ZD6474 to downregulate EGFR activation; however, inhibition of both EGFR activation and HIF-1α synthesis by ZD6474 varied temporally with different cell lines.

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Targeted delivery of endostatin using an antibody-endostatin fusion protein resulted in enhanced inhibition of tumor growth

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Endostatin can inhibit angiogenesis and tumor growth in mice. Factors limiting the utility of endostatin in humans include a short serum half-life that may necessitate frequent dosing and result in low concentrations at tumor sites. To increase efficacy, endostatin was combined with the targeting specificity of an anti-tumor antibody by producing antibody-endostatin fusion protein specific for the HER2/*neu* tumor antigen, in which murine endostatin was fused to C_H3 domain of human IgG3 antibody. While normal endostatin rapidly cleared from serum in mice ($t_{1/2}$ β: 38–225 min) and was degraded within 60 min (only 55% intact), anti-HER2/*neu* IgG3-endostatin remained stable for up to 96 hours (90% intact, $t_{1/2}$ β: 2410–2640 min). Antigen-specific targeting of HER2/*neu* IgG3-endostatin was evaluated in a BALB/c mice implanted with CT26 tumors and CT26 tumors engineered to express the HER2/*neu* target antigen (CT26-HER2). Anti-HER2/*neu* IgG3-endostatin preferentially localized to CT26-HER2 tumors relative to CT26 tumors. Administration of anti-HER2/*neu* IgG3-endostatin to mice bearing both CT26 and CT26-HER2 showed preferential inhibition of CT26-HER2, compared to parental CT26 tumor contralaterally implanted within the same mice. Anti-HER2 /*neu* IgG3-endostatin also inhibited the growth of the human breast cancer SK-BR-3 xenografts in SCID mice. Anti-HER2/*neu* IgG3-endostatin inhibited tumor growth more effectively than endostatin, anti-HER2/*neu* IgG3 antibody, or than the combination of antibody and endostatin. CT26-HER2 tumors treated with endostatin fusion had lower blood vessel density and complexity than the well-branched vessels characteristic of the parental tumor CT26, suggesting that anti-tumor effects were dependent on antivascular effects of the endostatin domains. Thus, the enhanced effectiveness of anti-HER2/*neu* IgG3-endostatin results from its longer half-life, selective targeting and the presence of a fused antiangiogenic factor. Our findings provide clear evidence that targeted endostatin delivery may be superior to endostatin or anti-HER2/*neu* antibody given alone, or in combination.

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Rodent pharmacokinetics and antiangiogenic activity of a pyrimidopyrimidine dual KDR/FGFR antagonist

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Among factors identified as regulators of angiogenesis, VEGF and bFGF and their receptors are thought to play key roles in tumor angiogenesis. VEGF mediates endothelial cell proliferation and migration, tube formation and vascular permeability. VEGF mediates these effects via the endothelial cell tyrosine kinase receptors, KDR/Flk-1 and Flt-1. bFGF is a mitogen and differentiation factor as well as a potent inducer of angiogenesis. Pyrimidopyrimidine 4 is a potent, orally active, low molecular weight ATP-competitive antagonist of key receptor tyrosine kinases known to play essential roles in tumor angiogenesis. The molecule is a soluble analog of compound 1 which was identified by high-throughput screening for inhibitors of KDR tyrosine kinase activity (AACR 95th Annual Meeting 2004 abstract #2478, 2570). The molecule is active versus KDR/VEGFR-2 (IC_{50} = 44nM), FGFR-1 (IC_{50} = 29nM), and PDGFR (IC_{50} = 33nM). Compound 4 is selective against a number of other ser/thr and tyrosine kinases. Compound 4 inhibits VEGF- and bFGF-stimulated proliferation of HUVECs, but is relatively inactive in tumor cell proliferation assays. Additionally, compound 4 at doses ranging from 25 to 100 mg/kg twice per day orally, significantly inhibited the growth of neovasculature in both the VEGF- and bFGF-driven corneal pocket assay in C57/BL6 mice. Drug exposure after oral dosing in these studies demonstrated linear kinetics and no dose accumulation when comparing levels in single-dosed animals versus those chronically treated. Oral bioavailability in athymic nude mice and the Wistar rat has also been established.

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Adaphostin (NSC680410) down regulates VEGF expression in human umbilical vein endothelial cells under hypoxia

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Vascular endothelial growth factor contributes to tumor growth by stimulating new tumor blood vessels (angiogenesis). Agents that inhibit VEGF signaling and vascular endothelial cell growth are currently in development as novel anticancer therapies. Adaphostin (NSC680410), a tyrosine kinase inhibitor inhibits endothelial cell growth, tube formation and VEGF induced cell migration. Adaphostin induces endothelial cells apoptosis and increases VEGF secretion into the media at concentrations of 0.75–1uM. Under these conditions the VEGF expression remains unchanged as measured by real time PCR. Western blot analysis suggests a decrease in VEGF-R1 (Flt-1), and induction of Hsp70 whereas Hsp90 remains unchanged. Cell growth arrest is essential for maintenance of cell viability and decreased microenvironmental oxygen levels (hypoxia) are potent signals for G1 growth arrest. One characteristic feature of VEGF expression is its inducibility by hypoxia. Therefore we studied the effect of Adaphostin on endothelial cells under moderate hypoxia (0.1–1% oxygen). Our data indicates that Adaphostin down regulates VEGF expression and secretion under moderate hypoxia. Hypoxia protects endothelial cells from Adaphostin induced apoptosis by 30% and inhibits the induction of Hsp70 protein. Preliminary evidence of in-vivo modulation of VEGF has been demonstrated through measuring VEGF-mRNA in tumor samples. Animals treated with 10mg/kg twice daily with adaphostin showed a 40–50% decrease in tumor mRNA levels. This data is consistent with in vitro studies. In conclusion, our data suggests that hypoxia can protect endothelial cells from oxidative stress caused by Adaphostin but the underlying complex mechanism remains to be elucidated.