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Caspase-8 mutations in head and neck cancer confer resistance to death receptor-mediated apoptosis and enhance migration, invasion, and tumor growth

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ABSTRACT

Little is known regarding molecular markers in head and neck squamous cell carcinoma (HNSCC) that predict responsiveness to different therapeutic regimens or predict HNSCC progression. Mutations in procaspase-8 occur in 9% of HNSCC primary tumors, but the functional consequences of these mutations are poorly understood. In this study, we examined the impact of four, representative, HNSCC-associated procaspase-8 mutations on activation of the extrinsic apoptosis pathway, as well as cellular migration and invasion, and *in vivo* tumor growth. All four mutant proteins acted to potently inhibit activation of apoptosis following treatment with TRAIL or agonistic anti-Fas. In contrast to wild-type procaspase-8, the mutant proteins were not recruited to FADD following treatment with TRAIL or anti-Fas, but may be constitutively bound by FADD. Three of the four procaspase-8 mutants promoted enhanced cellular migration and invasion through matrigel, relative to that seen with the wild-type procaspase-8 protein. Procaspase-8 mutation also stimulated the growth of HNSCC xenograft tumors. These findings indicate that HNSCC-associated procaspase-8 mutations inhibit activation of the extrinsic apoptosis pathway and are likely to represent markers for resistance to therapeutic regimens incorporating death receptor activators. Moreover, procaspase-8 mutations may serve as markers of HNSCC tumor progression, as exemplified by enhanced migration, invasion, and tumor growth.

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1. Introduction

Head and neck squamous cell carcinoma (HNSCC), the 7th leading cause of cancer mortality worldwide, is most commonly detected as advanced locoregional disease at initial diagnosis (Jemal et al., 2009; Mehanna et al., 2010). Despite a treatment armamentarium of surgery, radiation, and chemotherapy (platinum- or taxane-based) for patients with advanced disease, roughly 50% of these individuals will recur within 5 years, and the overall 5-year survival rate is only 50–60% (Fung and Grandis, 2010). Complicating treatment efforts are the considerable toxicities and side effects associated with current standard of care. Patients treated with surgery, radiation, and/or chemotherapy typically suffer disfigurement, difficulties in swallowing and speaking, as well as other adverse toxicities (Fung and Grandis, 2010). Moreover, relatively little information is available about molecular markers that predict HNSCC progression or responsiveness/resistance to therapy, including the FDA-approved EGFR monoclonal antibody cetuximab (Bonner et al., 2010). Thus, endeavors to rationally stratify HNSCC patients for different therapeutic regimens have proven difficult. In this regard, recent sequencing of HNSCC tumors has sought to determine the mutational landscape in this disease, with the hope of identifying markers that would be useful for prognostication and patient stratification.

Stransky et al. (Stransky et al., 2011) and Agrawal et al. (Agrawal et al., 2011) reported whole exome sequencing of 74 and 32 HNSCC primary tumors, respectively. Sequences encoding procaspase-8 were found to be mutated in 7% of patients. Similarly, mutations in procaspase-8 coding sequences have been identified in 9% of HNSCC patients ($n = 310$ tumors) in The Cancer Genome Atlas (TCGA; mutation data obtained from the cBio portal (Cerami et al., 2012)). Genomic analysis has revealed that the procaspase-8 mutations occurring in HNSCC are associated with fewer total and focal copy number alterations, and commonly are found in conjunction with HRAS mutations (Pickering et al., 2013). However, the impact of HNSCC-associated procaspase-8 mutations on disease progression, and the mechanism of action of the mutant proteins, remain unknown.

Wild-type caspase-8 protein plays a key role in the extrinsic, or death receptor-mediated, apoptosis pathway (Fulda, 2009). The procaspase-8 zymogen consists of a prodomain containing two death effector domains (DEDa and DEDb), a large subunit, and a small subunit (Degterev et al., 2003; Zhao et al., 2010). Following stimulation of a cell surface death receptor, procaspase-8 is recruited to the death-inducing signaling complex (DISC) affixed to the cytoplasmic region of the receptor (Dickens et al., 2012; Martin et al., 1998; Muzio et al., 1998; Schleich et al., 2012; Yang et al., 1998). The recruited procaspase-8 then undergoes processing to yield active caspase-8, which subsequently cleaves and activates the executioner caspase, caspase-3. Thus, caspase-8 mediates activation of the extrinsic apoptosis pathway by ligands in the tumor necrosis factor (TNF) family of death ligands, including Fas ligand and TRAIL. In addition, in response to TNF, caspase-8 is involved in mediating activation of anti-apoptotic NF-κB (Chaudhary et al., 2000). Complexes of caspase-8 with FADD and c-FLIP$_L$ also act to inhibit RIPK-dependent necrosis (Oberst et al., 2011). More recently, non-protealytic functions of caspase-8 have been identified, including promotion of cell adhesion, motility, and metastasis of neuroblastoma and lung cancer cell lines via Src-mediated phosphorylation of Tyr380 (Barbero et al., 2008; Finlay and Vuori, 2007; Helfer et al., 2006; Senft et al., 2007; Stupack et al., 2006).

Mutation of procaspase-8 has also been reported in hepatocellular carcinoma (Soung et al., 2005b), gastric carcinoma (Soung et al., 2005a), and colorectal carcinoma specimens (Kim et al., 2003), suggesting an important consequence of these mutations for the growth or survival of these tumors. In HNSCC, an early report identified a frameshift mutation in procaspase-8 as producing a fusion protein antigen recognized by cytotoxic T lymphocytes (Mandruzzato et al., 1997). Ando et al. subsequently identified a missense procaspase-8 mutation (G325A) in a HNSCC cell line (T3M-1 CI-10) that activates NF-κB signaling to a greater degree than wild-type caspase-8 when exogenously expressed in cells (Ando et al., 2013). Pickering et al. have identified 7 HNSCC cell lines that harbor procaspase-8 mutations and report that these cell lines tend to be more tumorigenic in athymic mice than nonsyngenic HNSCC cell lines harboring wild-type procaspase-8 (Pickering et al., 2013).

In this study we sought to determine the impact of procaspase-8 mutations identified in primary HNSCC tumors on activation of the extrinsic apoptosis pathway, as well as cellular migration and invasion, and *in vivo* tumor growth. Expression of 4 representative procaspase-8 mutations profoundly inhibited caspase activation and cell death following treatment with TRAIL or agonistic anti-Fas antibody. The mutant proteins also differed from wild-type procaspase-8 in their recruitment to DISC components. We further determined that 3 of 4 mutants promoted cellular migration and invasion, and a selected mutant enhanced HNSCC xenograft tumor growth. Collectively, our findings provide experimental evidence that procaspase-8 mutations associated with HNSCC serve as markers of resistance to death ligands, and may have value in predicting locoregional invasion or distant metastasis in HNSCC.

2. Materials and methods

2.1. Cells and reagents

The human HNSCC cell lines UM-SCC-22A, UM-SCC-22B, UM-SCC-1, 1483, Cal33, UPCI:SCC090, UM-SCC-47, and UD-SCC-2 (Lin et al., 2007), and HeLa cells were maintained in DMEM containing 10% heat-inactivated fetal bovine serum (FBS) and 100 units/mL of penicillin/streptomycin (Gibco Life Technologies). Jurkat cells were cultured in RPMI 1640 supplemented with 10% FBS and antibiotics. Cell lines were genotypically validated using the AmpFLSTR Profiler Plus Amplification Kit (A&B Applied Biosystem). TRAIL was obtained from Peprotech (310-04). *Pfx*50™ DNA Polymerase (12355-012) and MAX Efficiency® DH5α™ Competent Cells

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