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Signaling by cell surface death receptors: alterations in head and neck cancer

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Abstract

Cell surface death receptors are members of the tumor necrosis factor receptor (TNFR) superfamily and mediate signals leading to the induction of apoptosis or necroptosis, as well as NF-κB-mediated cell survival. These biochemical processes play key roles in cell growth, development, tissue homeostasis, and immune responses. The downstream signaling complexes activated by different death receptors can differ significantly and are subject to multiple, distinct regulatory mechanisms. Dysregulation of signaling by the TNFR superfamily contributes to a variety of pathologic conditions, including defective immune responses and cancer. Caspase-8 signaling is important for mediating death receptor signals leading to either apoptosis or NF-κB activation. By contrast, inactivation of caspase-8 or loss of caspase-8 expression shifts death receptor signaling to the necroptosis pathway. Notably, the gene encoding caspase-8 is mutated in roughly ten percent of head and neck cancers. These findings support the hypothesis that alterations in the biochemical pathways mediated by death receptors have important consequences for the development of head and neck, and possibly other, cancers.

Keywords

Death receptor; NF-κB; caspase-8; HNSCC; TNFR; TRAIL

1. Introduction

Cell Surface death receptors are a distinct subset of the tumor necrosis factor receptor (TNFR) superfamily members that are characterized by the presence of an intracellular death domain (DD) (Ashkenazi 2002). Signaling by death receptors involves the intracellular assembly of a number of protein complexes. The composition of these protein complexes dictates whether the cell will proceed to undergo apoptotic cell death, necroptotic cell death,

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or NF-κB-mediated gene expression and cell survival. As described in this review, the formation of death receptor-mediated signaling complexes is dependent on a variety of biochemical events, including “lock and key” protein-protein interactions, protein phosphorylation, protein ubiquitination, and proteolytic processing. In addition, death receptor signaling is negatively regulated by several endogenous proteins. In this chapter, we will describe the different protein complexes that are formed in response to death receptor stimulation and the biochemical processes that ensue. Further, we will characterize the genetic alterations in these signaling complexes and pathways that have been discovered in head and neck cancer, a common and lethal malignancy. The implications of these genetic alterations will be discussed.

2. Death receptor-mediated apoptosis signaling

Apoptosis is a form of programmed cell death in which internal or external stimuli prompt the activation of a cascade of caspase proteases that ultimately results in characteristic cellular changes, including membrane blebbing, DNA fragmentation, and RNA degradation (Atkin-Smith and Poon 2017). The importance of this process cannot be understated as genetic ablation of several pathway components can lead to profound developmental defects and even embryonic lethality in model organisms (Meier, Finch, and Evan 2000; Varfolomeev et al. 1998; Kaiser et al. 2011; Yeh et al. 1998). Two primary apoptosis signaling pathways have been described, the intrinsic apoptosis pathway and the extrinsic pathway (Hengartner 2000). The intrinsic pathway can be initiated by a multitude of different stimuli, including ionizing radiation, chemotherapy drugs, hypoxia, loss of membrane integrity, and viral infection. This pathway is characterized by the release of cytochrome c from the mitochondria and formation of an apoptosome complex consisting of cytochrome c, caspase-9, and the adaptor protein Apaf-1 (Hengartner 2000). The extrinsic apoptotic pathway, on the other hand, is mediated by cell surface death receptors. Death receptor-mediated apoptosis can be subdivided into two major categories: apoptosis induced by the death ligand tumor necrosis factor (TNF) and apoptosis induced by the death ligands Fas ligand (FasL) or TNF-related apoptosis-inducing ligand (TRAIL). Both of these pathways involve the binding of a death ligand member of the TNF superfamily to a member of the TNFR superfamily. The binding of death ligand to a cognate death receptor leads to activation of the death receptor and subsequent formation of an intracellular death-inducing signaling complex (DISC) (Figure 1A and 1B).

Apoptosis induction by death receptor signaling is central to the killing of malignant cells by cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. Upon activation, CTLs and NK cells induce the expression of death ligands on their surface. These death ligands interact with cell surface death receptors present on the surface of abnormal cells, including cancer cells, initiating cell death in the target cell.

DISC formation

TNF-induced signaling resulting from binding to TNF-R1 leads to the formation of either of two distinct protein complexes. Complex I promotes downstream NF-κB signaling, and will be discussed in greater detail in a later section. Complex II is a DISC that contains the TNF

receptor-associated death domain protein (TRADD), Fas-associated via death domain (FADD), and the zymogen form of the initiator caspase, procaspase-8 (Brenner, Blaser, and Mak 2015). TRADD is a death domain-containing protein that binds to death domains in the cytoplasmic region of ligand-bound TNF-R1 (Hsu, Xiong, and Goeddel 1995). TRADD serves to recruit the adaptor protein, FADD. Interestingly, complex II is the only form of DISC that contains TRADD, as both the Fas and TRAIL receptors interact directly with FADD. FADD, which is common to all DISCs, contains two domains: a C-terminal death domain (DD) that interacts with TRADD and a N-terminal death effector domain (DED) that recruits and binds one of the DEDs present in the prodomain of procaspase-8 (Carrington et al. 2006).

The mechanism of FasL/TRAIL-induced cell death involves fewer steps than TNF-induced cell death. In this case, apoptosis is initiated by FasL binding to Fas receptor or TRAIL binding to either the DR4 or DR5 receptors (Ashkenazi and Dixit 1998). This leads to direct recruitment of FADD, which results in a conformational change in FADD that exposes its N-terminal DED, allowing for the binding of procaspase-8 to the DISC (Riley et al. 2015).

Caspase activation

During both TNF- and FasL/TRAIL-induced cell death, the formation of the DISC leads to the activation of caspases which are critical to the regulation and execution of apoptosis (Taylor, Cullen, and Martin 2008; Lavrik, Golks, and Krammer 2005; Man and Kanneganti 2016; Shalini et al. 2015). Caspases are cysteine proteases that cleave substrate proteins following aspartic acid residues. There are two categories of caspases: initiator caspases and effector caspases. In the case of death receptor-mediated apoptosis, the primary initiator of the caspase cascade is procaspase-8. A related caspase, procaspase-10, can also serve as the initiator caspase in some cases of death receptor-induced apoptosis (Wang, Chun, et al. 2001). Procaspase-8 contains two tandemly encoded N-terminal DEDs (located in the prodomain of the zymogen protein), a large catalytic subunit (p18), and a small catalytic subunit (p10) (Blanchard et al. 1999).

Oligomerization of procaspase-8 at the DISC occurs via a process that leads to elongated chains of procaspase-8 proteins, with the second DED of one procaspase-8 recruiting the first DED of another procaspase-8 molecule (Dickens et al. 2012). The formation of these multimeric chains leads to activation of the zymogen via autocleavage at two locations (Chang et al. 2003; Oberst et al. 2010). First, procaspase-8 present in the DISC undergoes proteolytic cleavage in the linker region that separates p18 and p10. The newly formed p10 remains bound to the DISC through its association with p18. A second cleavage event occurs between the second DED and p18, resulting in the release of the active caspase-8 heterotetramer containing two p18 subunits and two p10 subunits. Once processed and activated, caspase-8 cleaves and activates downstream executioner caspases, of which, the major executioner caspase present in cells is caspase-3 (Stennicke et al. 1998). The executioner caspases directly cleave a broad array of cellular substrate proteins leading to activation of the various processes involved in apoptotic cell death (Walsh et al. 2008; Slee, Adrain, and Martin 2001).

3. Negative regulators of death receptor-mediated apoptosis signaling

Apoptosis is a critically important process for development, tissue homeostasis, and immune responses, and is, therefore, highly regulated (Meier, Finch, and Evan 2000; Opferman 2008; Henson and Hume 2006). Aberrant dysregulation of this process is associated with numerous pathologic conditions and is one of the hallmarks of cancer (Carson and Ribeiro 1993; Cotter 2009; Hanahan and Weinberg 2011). As discussed above, several factors are involved in triggering and executing apoptosis signaling. Not surprisingly, there are also several layers of negative regulation, mediated by a variety of cellular proteins, including decoy receptors, inhibitor of apoptosis proteins (IAPs), and cellular FLICE-like inhibitory protein (c-FLIP) (Figure 1A and 1B). The mechanisms of apoptosis inhibition mediated by these proteins are discussed below.

Decoy Receptors

Decoy death receptors are capable of binding death ligands, but fail to initiate apoptosis signaling in response to this event (Ashkenazi 2002; Ashkenazi and Dixit 1999). Four distinct genes encoding decoy death receptors have been identified in the human genome, and code for: decoy receptor 1(DcR1), decoy receptor 2 (DcR2), decoy receptor 3 (DcR3), and osteoprotegerin. DcR1 and DcR2 both contain an extracellular TRAIL binding domain and a transmembrane domain. However, while DcR2 contains a truncated death domain in the cytoplasmic region, DcR1 completely lacks a death domain. DcR3 is a soluble decoy receptor that competes with the Fas receptor for binding to FasL. Osteoprotegerin has primarily been studied as a decoy receptor for RANKL, although it has also been shown to bind TRAIL. Little is known about the physiological impact of decoy receptor expression on apoptotic signaling, but the overarching hypotheses are that the decoy receptors compete with functional death receptors for binding to death ligands or that they act as dominant-negative inhibitors of death receptor signaling. By either mechanism, the decoy receptors inhibit activation of apoptosis signaling.

IAPs

IAPs were first discovered in the insect virus, baculovirus, but several homologs have since been found in mammalian genomes (Seshagiri and Miller 1997). The human genome encodes eight IAP proteins, three of which are well characterized and known to negatively regulate apoptosis signaling: XIAP, cIAP1, and cIAP2 (Srinivasula and Ashwell 2008; Gyrd-Hansen and Meier 2010; Silke and Meier 2013). All IAPs contain at least one baculovirus IAP repeat (BIR) domain, which binds zinc and facilitates protein-protein interactions. XIAP, cIAP1, and cIAP2 each contain three BIR domains, as well as a RING domain with ubiquitin ligase activity. cIAP1 and cIAP2 also contain a caspase-associated recruitment domain (CARD), which can regulate the ubiquitin ligase activities of these proteins by preventing RING dimerization and E2 recruitment (Lopez et al. 2011). Despite structural similarities, XIAP, cIAP1, and cIAP2 have been shown to inhibit apoptosis through very different mechanisms.

The BIR domains of XIAP are known to directly inhibit caspase activity. The BIR2 domain of XIAP physically associates with the substrate binding sites of caspase-3 and caspase-7,

leading to inhibition, while the BIR3 domain of XIAP inhibits activated caspase-9 through an allosteric mechanism (Riedl et al. 2001; Suzuki et al. 2001; Datta et al. 2000; Srinivasula et al. 2001). The mechanisms of action of cIAP1 and cIAP2 are less clear. Indeed, cIAP1 and cIAP2 have also been shown to bind caspases, but these interactions are not sufficient for significant inhibition of caspase activity (Eckelman and Salvesen 2006). Instead, cIAP1 and cIAP2 are thought to regulate apoptosis by utilizing the ubiquitin ligase activity associated with the RING domain. cIAP1 and cIAP2 have been shown to promote the ubiquitination of caspase-3 and caspase-7 as well as second mitochondria-derived activator of caspases (SMAC), a negative regulator of XIAP (Hu and Yang 2003; Choi et al. 2009). Furthermore, cIAP1 and cIAP2 are also found in the TNF-induced complex I and are critical for the activation of NF-κB at the expense of apoptosis. Activation of NF-κB via this mechanism is primarily due to ubiquitination of receptor interacting serine/threonine protein kinase 1 (RIPK1) (Bertrand et al. 2008). The downstream implication of this event will be discussed in a subsequent section. Regardless of the exact mechanisms by which these three IAPs inhibit apoptosis, they seem to have some functional redundancy as genetic ablation of any one of the three genes does not result in severe defects in model organisms (Silke and Vaux 2015).

c-FLIP

The FLICE-like inhibitory protein (FLIP) protein was also originally discovered as a viral gene product (v-FLIP) (Thome et al. 1997). The human gene (*c-Flip*) is generally expressed as two major isoforms, c-FLIP$_S$, and c-FLIP$_L$, that result from alternative splicing (Safa 2012). c-FLIP$_S$ is relatively short and is comprised of only two DED domains and a short C-terminal tail. By contrast, c-FLIP$_L$ is similar in structure to caspase-8, and contains two tandemly encoded N-terminal DEDs followed by a large (p20) and a small (p12) subunit (Safa 2012). Importantly, c-FLIP$_L$ has several amino acid differences in the large and small subunits that distinguish it from caspase-8 and render it catalytically inactive. c-FLIP$_S$ strictly acts as an inhibitor of procaspase-8 activation at the DISC by impeding procaspase-8 oligomerization. Interestingly, the function of c-FLIP$_L$ is thought to be two-fold. Low levels of c-FLIP$_L$ have a stimulatory effect on apoptosis, while high levels of c-FLIP$_L$ have an inhibitory effect on apoptosis (Chang et al. 2002; Hughes et al. 2016). The precise mechanism underlying this dose-dependence is not completely understood, but the current working model is that low levels of c-FLIP$_L$ facilitate heterodimerization with procaspase-8 and activate the zymogen independent of autocleavage. Conversely, high levels of c-FLIP$_L$ may inhibit multimeric oligomerization of procaspase-8 similar to the mechanism of inhibition by c-FLIP$_S$.

4. Death receptor-mediated necroptosis signaling

It was generally thought that there were two forms of cell death: caspase-dependent apoptosis and a more catastrophic form of passive cell death called necrosis. More recently, however, it has been discovered that cells can undergo a caspase-independent form of programmed cell death, termed necroptosis (Han, Zhong, and Zhang 2011; Chan, Luz, and Moriwaki 2015; Grootjans, Vanden Berghe, and Vandenabeele 2017; Vanden Berghe et al. 2014; Vandenabeele et al. 2010). Importantly, this process has been shown to occur as a

result of death receptor stimulation. Necroptosis is characterized by the formation of a complex called the necrosome that consists of the proteins RIPK1, RIPK3, and mixed lineage kinase like (MLKL) (Figure 2A and 2B).

As discussed above, death receptor stimulation can lead to the formation of a DISC. In most cases, the activation of caspase-8 that follows DISC formation results in the proteolytic cleavage and inactivation of RIPK1 and RIPK3 (Ofengeim and Yuan 2013; Declercq, Vanden Berghe, and Vandenabeele 2009; Lin et al. 1999; Feng et al. 2007). Interestingly, procaspase-8/c-FLIP$_L$ heterodimers are also capable of inactivating RIPK1, driving signaling toward apoptosis (Micheau et al. 2002). This suggests a potential mechanism by which low c-FLIP$_L$ concentrations enhance apoptotic potential. However, under conditions where caspase-8 activity is missing, RIPK1 and RIRK3 are free to aggregate in microfilaments via RIP homotypic interaction motif (RHIM)-dependent binding (Li et al. 2012). The formation of these complexes leads to the autophosphorylation and transphosphorylation of RIPK1 and RIPK3 (Cho et al. 2009). Phosphorylation of RIPK3 on Ser227 enables recruitment of MLKL to the necrosome complex (Moriwaki and Chan 2013; Zhao et al. 2012). Once recruited, MLKL is phosphorylated by RIPK3 on Thr357 and Ser358, leading to MLKL activation (Wang et al. 2014). Activated MLKL proteins form oligomers that are translocated to intracellular membranes and the plasma membrane, where they bind directly to phosphatidylinositol lipids and cause a loss of membrane integrity (Wang et al. 2014). In this sense, MLKL is the primary executioner of necroptosis.

5. Death receptor-mediated NF-κB signaling

While death receptor activation is known to lead to cell death by apoptosis or necroptosis, as discussed above, it also has the potential to promote cell survival. Accordingly, death receptor activation can be thought of as a two-sided coin, where cell survival signals downstream of death receptors are primarily driven by activation of the transcription factor, NF-κB. There are two primary pathways of NF-κB activation: the canonical pathway which is characterized by the formation of p65/p50 heterodimers, and the noncanonical pathway which is characterized by formation of RelB/p52 heterodimers (Jost and Ruland 2007; Karin, Yamamoto, and Wang 2004; Gerondakis et al. 2014). The canonical pathway is known to be activated by death receptor signaling, while the noncanonical pathway is activated by other signals. Here, we will focus on activation of the canonical NF-κB pathway by death receptors (Figure 3A and 3B).

TNF-induced NF-κB signaling

As mentioned earlier, ligation of TNF to TNF-RI can lead to the formation of a pro-survival complex termed complex 1. Complex 1 consists of TNF, TNF-R1, TRADD, RIPK1, TNF receptor-associated factor 2 (TRAF2), and the cIAPs (Brenner, Blaser, and Mak 2015). In sequential order, TNF binding to TNF-R1 leads to the recruitment of TRADD, which binds to the DD of TNF-R1 via its own DD and signals the recruitment of RIPK1 to the complex (Hsu, Xiong, and Goeddel 1995; Hsu et al. 1996). The formation of the TNF-R1/TRADD/RIPK1 complex facilitates the recruitment of TRAF2, which subsequently binds the cIAPs (Park et al. 2000; Vince et al. 2009; Zheng et al. 2010). Recruitment of the cIAPs to complex

1 results in the ubiquitination of RIPK1 at residues K11, K48, and K63 (Bertrand et al. 2008; Li et al. 2006; Ea et al. 2006).

Formation of complex 1 and ubiquitination of RIPK1 leads to the recruitment of both the heterotrimeric IKK complex, consisting of IKKα, IKKβ and NF-κB essential modulator (NEMO), and TGF-β activating kinase 1 (TAK1) (Ea et al. 2006). NEMO binds directly to the K63 polyubiquitin chains on RIPK1, while TAK1 associates with the complex through its interactions with the ubiquitin binding protein, TAK1 associating subunit 2 (TAB2) (Laplantine et al. 2009; Yoshikawa et al. 2009; Kanayama et al. 2004). TAK1 phosphorylates and activates IKKβ, which in turn phosphorylates the inhibitor of NF-κB, IκB, (Wang, Deng, et al. 2001). IκB normally functions to bind and immobilize the p65/p50 heterodimer in the cytoplasm (Baeuerle and Baltimore 1988b; Baeuerle and Baltimore 1988a). However, the phosphorylation of IκB by IKKβ signals the degradation of IκB by the proteasome, thereby allowing the p65/p50 heterodimer to translocate to the nucleus and drive gene expression (Mercurio et al. 1997).

FasL/TRAIL-induced NF-κB signaling

While potent activation of NF-κB signaling by TNF has long been known, the mechanism underlying NF-κB activation by FasL/TRAIL has only been described more recently. Due to the lack of TRADD recruitment to the receptors for FasL or TRAIL, FasL/TRAIL-induced NF-κB activation differs significantly from that induced by TNF. The activation of FasL/TRAIL-induced NF-κB activation is mediated by the formation of a cytoplasmic complex called the FADDosome (Henry and Martin 2017; Kreuz et al. 2004; Imamura et al. 2004; Cullen et al. 2013; Hartwig et al. 2017). The major components of the FaDDosome are FADD, procaspase-8, and RIPK1, all of which are critical for NF-κB activation in response to FasL/TRAIL (Henry and Martin 2017; Kreuz et al. 2004). Procaspase-10, c-FLIP$_L$, and TRAF2 are also commonly found in this complex, but the functional importance of these factors is less clear (Henry and Martin 2017). Interestingly, caspase-8 activity is not required for formation or function of the FADDosome, suggesting that procaspase-8 acts as a scaffold in this context (Henry and Martin 2017; Kreuz et al. 2004). Formation of the FADDosome leads to the ubiquitination of RIPK1 and the recruitment of both the IKK and TAK1/TAB1/TAB2 complexes (Henry and Martin 2017). NF-κB activation is accomplished through the phosphorylation of IκB by IKKβ and subsequent degradation of IκB, as described above.

6. Alterations of death receptor signaling in cancer

The recent publication by The Cancer Genome Atlas (TCGA) describing the genomic landscape of 279 head and neck squamous cell carcinoma (HNSCC) tumors has revealed several previously unanticipated alterations in this devastating disease (Cancer Genome Atlas 2015). Indeed, multiple alterations in the death receptor signaling network have been discovered (Cancer Genome Atlas 2015; Pickering et al. 2013) (Table 1). While these genomic studies are informative and hypothesis-generating, more work is needed to fully understand the impact of the identified alterations on cancer initiation and aggressiveness. The following analysis is based on mutation and copy number analysis (CNA) data from an

expanded cohort of 504 HNSCC cases released by the TCGA on cbioportal.org (Gao et al. 2013; Cerami et al. 2012).

Death ligands and receptors

Of the known death ligands and receptors, *TNFSF10* (encoding TRAIL), is the only gene found to be significantly altered in HNSCC. *TNFSF10* is located on chromosome 3q26 in close proximity to *PIK3CA*, *TP63*, and *SOX2*, and is amplified in approximately 20% of HNSCC cases. The *PIK3CA* gene encodes the p110α catalytic subunit of phosphatidylinositol 3-kinase (PI3K). Not surprisingly, *TNFSF10* (and *PIK3CA*) is also frequently amplified in cancers that are known to be driven, in large part, by PI3K, including lung squamous cell, breast, ovarian, esophageal, and cervical cancers. While *TNFSF10* is the only death receptor or ligand commonly altered in HNSCC, alterations of other death receptors and ligands are observed in other malignancies, such as amplification of *TNFRSF1A* (encoding TNF-R1) in >20% of testicular germ cell tumors and deletion of *TNFRSF10A* and *TNFRSF10B* (encoding DR4 and DR5, respectively) in >15% of prostate adenocarcinoma tumors.

DISC components

The most common alterations in components of the DISCs that are found in HNSCC are amplification of *FADD* (encoding FADD) and mutation of *CASP8* (encoding procaspase-8) in 25% and 10% of cases, respectively. It should be noted that *FADD* is found on the same amplicon on chromosome 11q13 as the gene encoding the well-known oncogene, *CCND1* (encoding cyclin D1). Mutations in *CASP8* are found throughout the length of the protein coding sequence, with no obvious hot spots. Interestingly, these alterations in *CASP8* and *FADD* are significantly mutually exclusive ($p<0.001$), suggesting that either they have a similar impact in HNSCC or they are synthetically lethal. *FADD* has also been found to be amplified in >10% of esophageal, breast, lung squamous cell, and bladder cancers, while there are no other tumor types with >10% incidence of *CASP8* mutations.

NF-κB signaling components

As mentioned above, *FADD* and *CASP8*, which are important components of the NF-κB activating FADDosome, are commonly amplified and mutated, respectively, in HNSCC. In addition to these two alterations, *BIRC2* and *BIRC3* (encoding cIAP1 and cIAP2, respectively) are amplified in approximately 6% of HNSCC cases. Because these genes are directly adjacent to each other on chromosome 11q22, they are always co-amplified. Genes encoding several downstream components of NF-κB signaling are also altered in HNSCC, including amplification or mutation of *IKBKB* (encoding IKKβ) in 5% of cases, and amplification of *RELA* (encoding the p65 subunit of NF-κB) in 5% of cases, among others. Although not statistically significant, many of the alterations in genes for the NF-κB signaling components located downstream of RIPK1 ubiquitination show a small amount of mutual exclusivity, resulting in 22% of HNSCC cases harboring gene alterations in *MAP3K7*, *TAB1*, *TAB2*, *IKBKG*, *CHUK*, *IKBKB*, *NFKBIA*, *RELA*, or *NFKB1*.

7. Conclusions

Signaling by cell surface death receptors is critically important for growth, development, and tissue homeostasis. In addition, numerous studies highlight the central role of death receptor signaling in immune function (Opferman 2008; Chan, Luz, and Moriwaki 2015; Davidovich, Kearney, and Martin 2014). Apoptosis induction by death receptor signaling is critical to the killing of malignant target cells by CTLs and NK cells. Moreover, recent work has shown that NF-κB activation in target cells via death receptor signaling can lead to induced expression of pro-inflammatory cytokines, resulting in the recruitment of additional immune cell populations to the tumor microenvironment (Hartwig et al. 2017; Cullen et al. 2013).

Caspase-8 is a central component of several of the protein complexes that mediate signaling by death receptors. The high frequency of caspase-8 mutations that are found in HNSCC (10% of cases) raises questions regarding the role of these mutations in promoting the development of this disease (Cancer Genome Atlas 2015). Characterization of a subset of HNSCC-associated procaspase-8 mutations has shown that the mutant proteins reduce the sensitivity of HNSCC cells to apoptosis induction by TRAIL (Li et al. 2014). Loss of caspase-8-mediated apoptosis in tumor cells from the oral mucosa could severely hamper the ability of CTL and NK cells to eliminate tumor cells. In addition, Ando *et al.* found that a procaspase-8 mutation derived from a HNSCC cell line promoted hyperactivation of NF-κB (Ando et al. 2013). In addition to inducing the expression of pro-survival genes, NF-κB is known to stimulate the production of a broad variety of cytokines and chemokines. The production of these factors may provide an autocrine stimulus for growth in HNSCC tumor cells. Alternatively, several of the factors induced by NF-κB are known to suppress the immune system and this immunosuppressive effect may further hamper the ability of the immune system to eliminate HNSCC tumor cells.

The loss of caspase-8 function is also known to shift signaling by death receptors from apoptosis signaling to necroptosis signaling, as described above. While the impact of procaspase-8 mutations on necroptosis signaling has not been reported, it is possible that cancer cells with inactivating caspase-8 mutations are more prone to death by necroptosis. Whether a shift from apoptosis to necroptosis contributes to HNSCC development is still unclear.

Moving forward, it will be important to fully characterize the impact that alterations in death receptor signaling components impose on cancer initiation and development, as well as the composition of the tumor microenvironment. Since these alterations are commonly observed in HNSCC, this cancer type represents an ideal model in which to perform important mechanistic studies and hypothesis testing. The data from these studies are likely to inform new treatment modalities for the subset of tumors harboring mutations in death receptor signaling components and identify biomarkers for currently available therapies.

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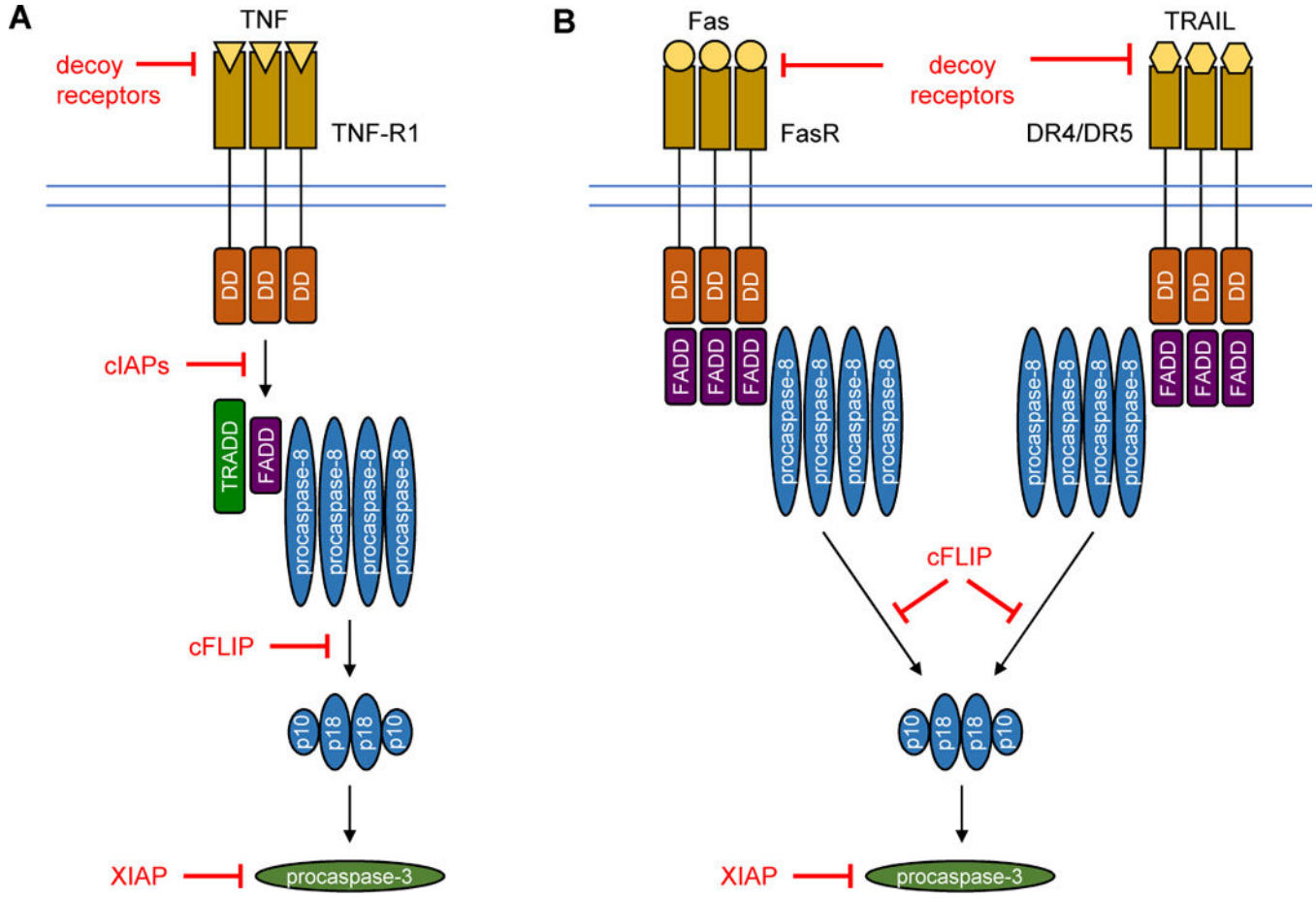

Figure 1. Death receptor-mediated apoptosis signaling

(**A**) Schematic of death receptor-mediated apoptosis in response to TNF. This pathway is highlighted by the formation of a soluble complex II containing TRADD, FADD and caspase-8 and subsequent activation of procaspase-8 by autocleavage. Inhibitors of this pathway are shown in red. (**B**) Schematic of death receptor-mediated apoptosis in response to FasL/TRAIL. Apoptosis induced by FasL/TRAIL is accomplished by formation of a DISC directly at the receptor. Inhibitors of this pathway are shown in red.

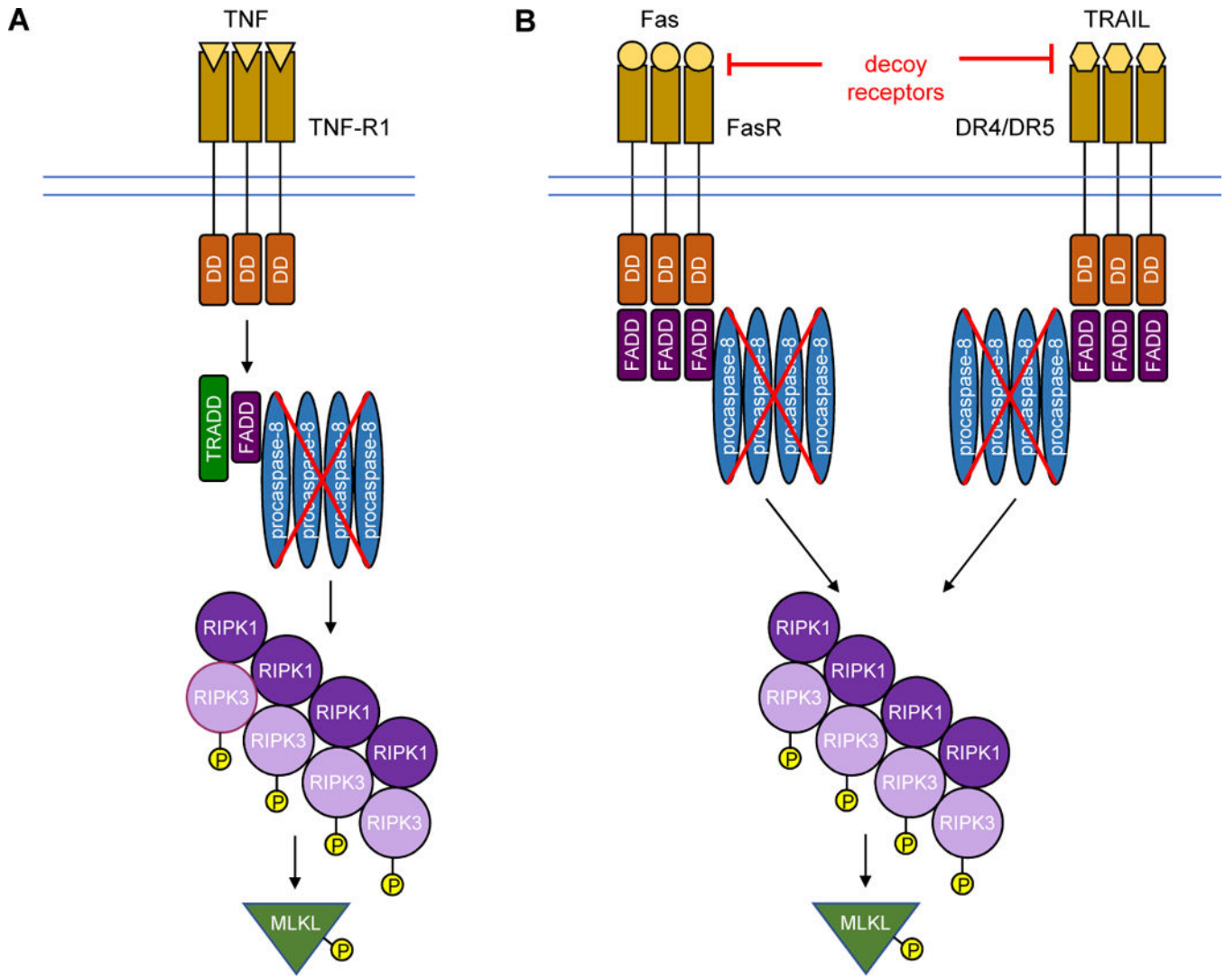

Figure 2. Death receptor-mediated necroptosis signaling

(**A**) Schematic of death receptor-mediated necroptosis in response to TNF. The lack of caspase-8 activity results in the accumulation of RIP kinases, the formation of RIP kinase-containing microfilaments, and the phosphorylation of MLKL. (**B**) Schematic of death receptor-mediated necroptosis in response to FasL/TRAIL. The induction of necroptosis in response to FasL/TRAIL occurs in a similar fashion to TNF-induced necroptosis.

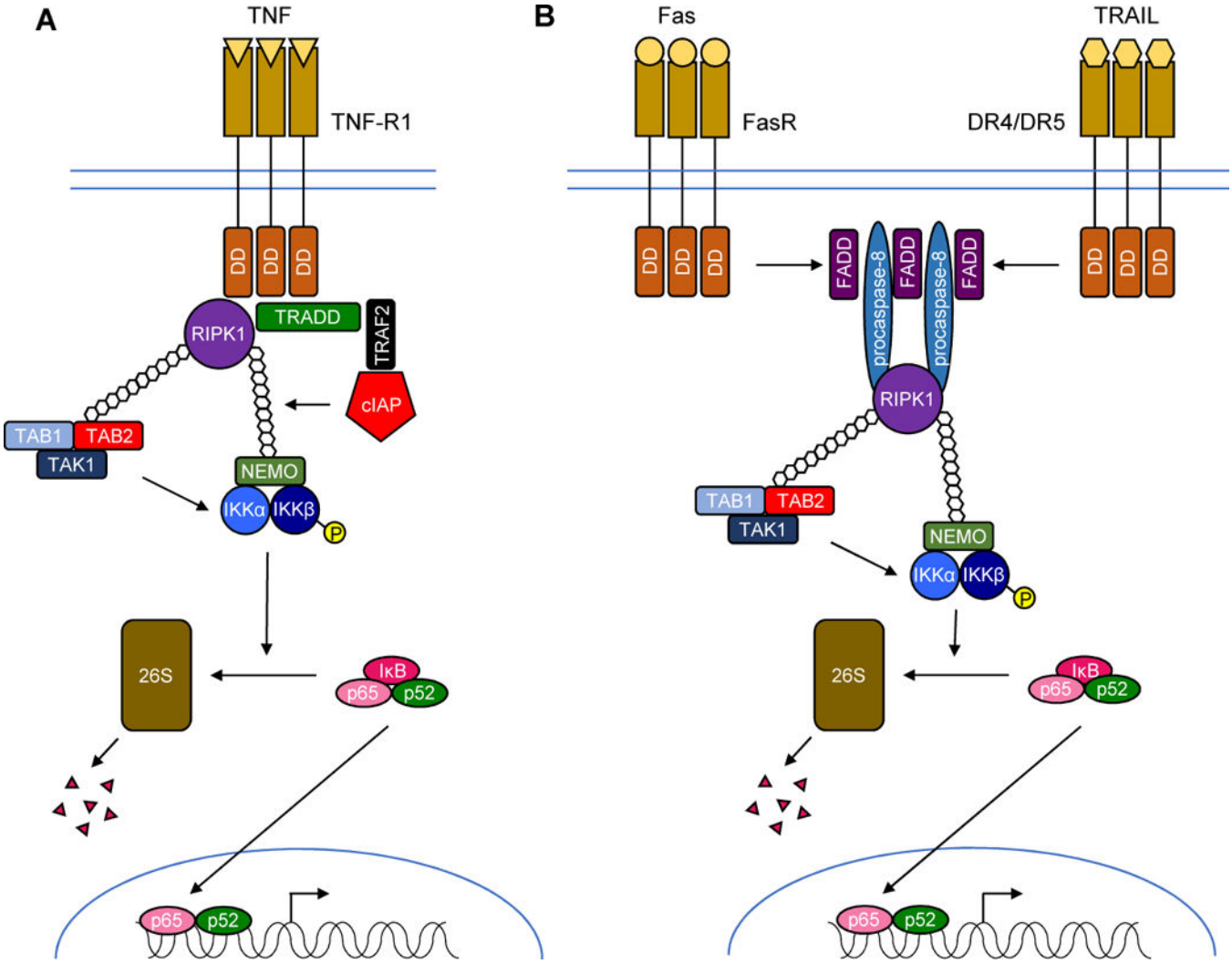

Figure 3. Death receptor-mediated NF-κB activation

(**A**) Schematic of death receptor-mediated NF-κB activation in response to TNF. Formation of complex I in response to TNF-R1 activation leads to the ubiquitination of RIPK1, activation of IKKβ by TAK1, degradation of IκB, and nuclear translocation of the NF-κB heterodimer. (**B**) Schematic of death receptor-mediated NF-κB activation in response to FasL/TRAIL. NF-κB activation by FasL/TRAIL ligands occurs through the formation of the FADDosome, ubiquitination of RIPK1, recruitment of the IKK and TAK1 containing complexes, and the eventual degradation of IκB.

Table 1

Death receptor pathway alterations in HNSCC

Gene	Protein	Location	Mutation	Amplification
Death Ligands and Receptors				
TNFSF10	TRAIL	3q26	<1%	19.7%
DISC components				
FADD	FADD	11q13	<1%	25.2%
CASP8	Procaspase-8	2q33	1.4%	10.1%
NF-|κB signaling components				
BIRC2	cIAP1	11q22	<1%	5.4%
BIRC3	cIAP2	11q22	<1%	5.8%
IKBKB	IKKβ	8p11	2.4%	2.6%
IKBKG	NEMO	Xq28	0%	3.6%
RELA	p65	11q13	<1%	4.8%