Apoptotic Signaling Pathways in Lung Cancer

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Tumor cells may respond to chemo- or radiotherapy by activation of several cellular signaling cascades that influence cell survival and cell death, including activation of cell cycle arrest, senescence or triggering of several cell death types (i.e., mitotic catastrophe, necrosis, or apoptosis).[1,2] However, tumor cells derived from solid tumors are often refractory to therapy or develop resistance during the treatment course. This is illustrated by non-small cell lung cancer (NSCLC), which shows a high degree of intrinsic resistance, and by small cell lung cancer (SCLC), which often develops resistance to treatment during the course of disease.[3] In part, defective apoptotic signaling may contribute to chemo-resistance and radiotherapy resistance.[1] However, it is also likely that other cell death modes (e.g., necrosis, autophagy, and mitotic catastrophe) and premature senescence are of equal importance for efficient tumor cell death in response to chemo- and radiotherapy.[2] In this article, we give a brief overview of the main apoptotic signaling pathways and their deregulation in lung cancer (LC), and we provide some examples of apoptosis-based therapies.

Apoptosis is distinguished by some morphological characteristics (i.e., plasma membrane blebbing, cell shrinkage, condensation/fragmentation of the chromatin, and disintegration of the cell into apoptotic bodies). All these characteristics are effects of selective proteolysis of proteins involved in cell signaling, DNA repair, or structural maintenance of DNA integrity, carried out by caspases, a group of cystein-aspartate enzymes.[4] Caspases are classified as initiator caspases (caspase-2, -8, -9, and -10 within human cells), which, upon activation, cleave and activate the second group, the effector caspases (mainly caspase-3, -6, and -7 within human cells), then perform selective proteolysis.[4]

Caspases are activated either by death receptor (DR) activation (extrinsic) or via mitochondrial release of apoptogenic proteins (e.g., cytochrome c, smac/DIABLO, and HtrA2/Omi) (intrinsic) (Figure 1). The signals propagated by the intrinsic pathway may also be generated in cell nuclei or lysosomes or within the endoplasmatic reticulum (Fig. 1).[5–7]

In the extrinsic caspase activation pathway, TNF superfamily ligands bind to DRs, causing oligomerization of DRs and recruitment of adaptor proteins via a death domain. In turn, adaptor proteins bind pro-caspase-8 via a death effector domain (DED) allowing pro-caspase-8 to be activated, an event that is critically dependent on the adaptor recruitment domain in the pro-caspase-8.[8] Active caspase-8 then either activates pro-caspase-3 directly or amplifies the signal through Bid-cleavage (Figure 1).[9] In the intrinsic pathway, apoptotic signals trigger increased mitochondrial outer membrane permeability (MOMP), followed by selective release of apoptogenic proteins from the mitochondrial inter membrane space to the cytosol (e.g., cytochrome c, Smac/DIABLO, and HtrA2/omi), all which promote caspase activation (Fig. 1).[9] Cytosolic cytochrome c forms a complex with apoptosis protease-activating factor 1 (Apaf-1) and dATP (i.e., the apoptosome), in which the dimerization of pro-caspase-9 occurs, allowing its activation into caspase-9. This is followed by pro-caspase-3 activation.[8] For these to efficiently result in apoptotic propagation, the concomitant alleviation of the caspase-blocking effect of inhibitor of apoptosis proteins (IAPs) is required. Hence, the release of Smac/DIABLO and HtrA2/omi, both which block IAPs and both which are released as a consequence of increased MOMP, leads to increased caspase-3 activity.[9] In part, MOMP is controlled by Bcl-2 family proteins, and the anti-apoptotic members Bcl-xL and Bcl-2 both inhibit MOMP. Accordingly, pro-apoptotic members such as Bak or Bax, both which are activated by some BH3-only proteins (Bid, Bim, Bad, PUMA, and NOXA), can promote MOMP.[10]

An important regulator of mitochondria-mediated signaling is the tumor suppressor p53. Thus, p53 may induce expression of pro-apoptotic proteins (e.g., Bax, PUMA, Apaf-1) and/or repress anti-apoptotic proteins, including Bcl-2, in response to DNA damage.[11] In addition, p53 can re-localize to cytosol and in the same way as BH3-only proteins trigger Bak and/or Bax activation.[12,13]

CASPASE ACTIVITY ADJUSTERS

Caspase activity can be restrained by inhibitor of apoptosis proteins (IAPs), by heat shock proteins (HSPs), or by changes in protein kinase signaling. Briefly, IAPs (cIAP-1,-2, XIAP, and survivin) cause a structural block within the substrate-binding pocket of caspases, which impede substrate binding and target the bound caspases for proteosomal deg-

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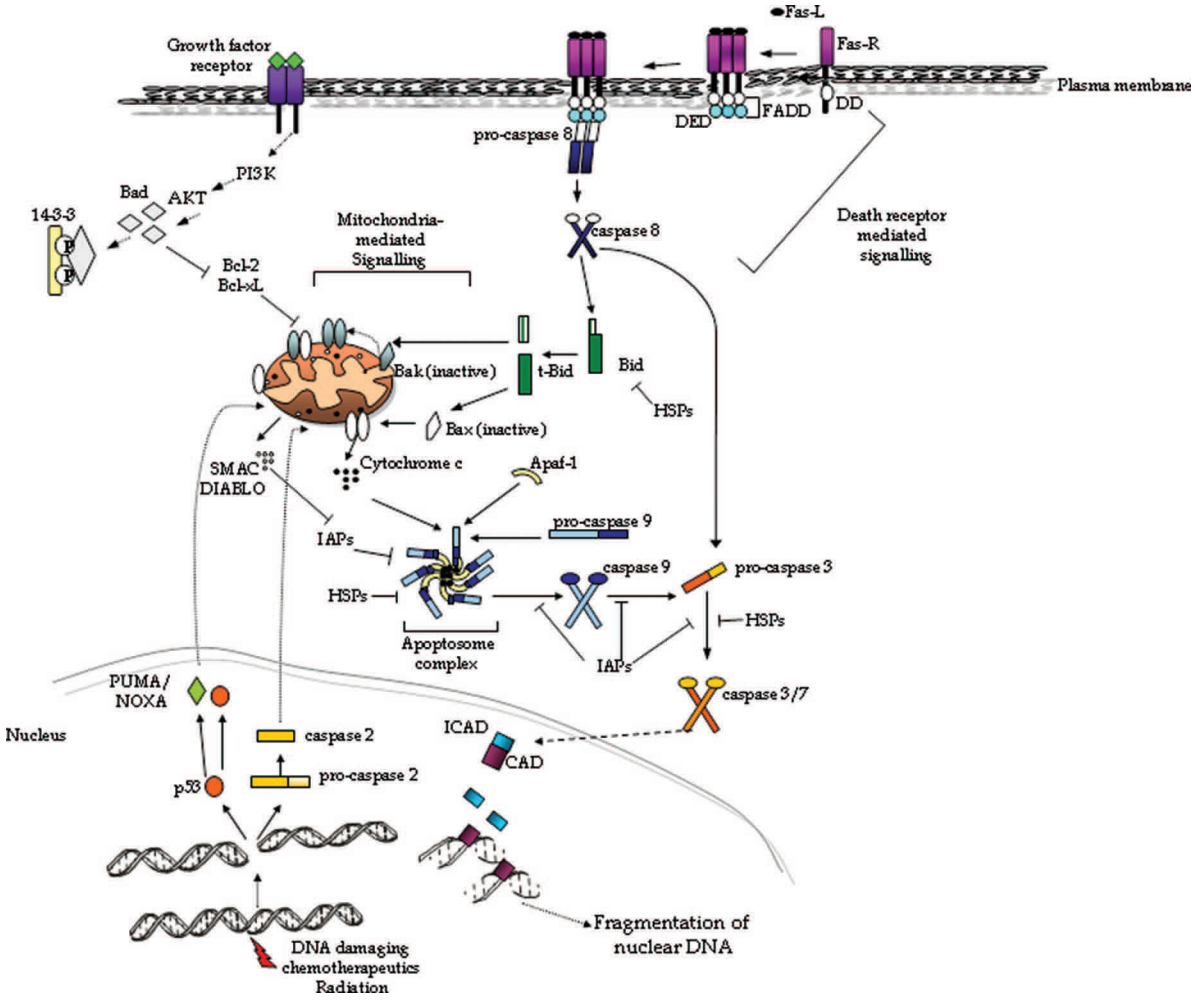

FIGURE 1. Apoptotic signaling pathways. Caspases are activated by extrinsic (death receptor-mediated) or intrinsic pathway (mitochondria-mediated) signaling. Death ligands bind to their receptors (Fas-L and Fas-R) and via death domain (*DD*) adaptor proteins (*FADD*) are bound. Via a death effector domain (*DED*), the adaptor protein recruits pro-caspase-8, which gets activated. Caspase-8 thereafter directly activates pro-caspase-3, which cleaves structural proteins and inhibitor of caspase-activated DNase (*ICAD*), resulting in free caspase-activated DNase (*CAD*), which causes fragmentation of nuclear DNA. To amplify the signal, caspase-8 may also cleave Bid into t-Bid, which can initiate mitochondria-mediated signaling. The mitochondria-mediated pathway results in increased mitochondrial outer membrane permeability (*MOMP*) and release of apoptogenic proteins to cytosol (cytochrome c and Smac/DIABLO). The Bcl-2 family proteins (Bcl-2, Bcl-XL, Bak, Bax, Bad, and Bid) in part control MOMP. Within cytosol, cytochrome c forms a complex together with Apaf-1 the apoptosome, in which pro-caspase-9 is activated. Activated caspase-9 then triggers pro-caspase-3 activation. At several levels, caspase processing and/or activity can be inhibited by inhibitor of apoptosis proteins (*IAPs*) or by heat shock proteins (*HSPs*). IAPs are antagonized by Smac/DIABLO. Apoptotic signaling is also influenced by growth factor receptor signaling (exemplified by the Akt-pathway), which blocks Bad function by inducing binding to 14-3-3 proteins. Apoptotic signals can also be initiated at other places within the cell, (exemplified by the cell nuclei), in which p53, PUMA, NOXA, and caspase-2 gets activated on DNA damage and transmit pro-apoptotic signals to mitochondria.

radation.[14] HSPs (HSP90, HSP70, HSP60, and HSP27) can block caspase activity through sequestration of cytochrome c, inhibition of Bid redistribution, or Akt dephosphorylation or by blocking Apaf-1–mediated pro-caspase-9 or -3 activation.[15]

The phosphatidylinositol 3-kinase (PI3-K)/Akt-dependent pathway and the Ras-activated mitogen-activated protein kinase (MAPK) pathway both influence apoptotic propensity.[16] Although Akt and MAPK/ERK mainly are activated by growth factors and inhibit pro-apoptotic signaling, the MAPKs p38 and JNK can also be activated in response to cellular stress (e.g., DNA damaging treatments) and be either pro- or anti-apoptotic depending on stimuli, duration, and cell type.[16] Both Akt and ERK may inhibit Bad or caspase-9 or antagonize Bim.[10,17,18] JNK is known to regulate cytochrome c release in part by promoting Bax /Bak activation or complex formation or by

inhibiting Bcl-2/Bcl-xL.[19–24] JNK also promotes expression of Bak, Bax, and Bim.[25] In a similar way, p38 controls apoptotic signaling by antagonizing or promoting the Bcl-2 family proteins.[26,27,28]

ALTERATIONS IN APOPTOTIC SIGNALING PATHWAYS IN LUNG CANCER

Loss of pro-caspase-8, FasL, or DRs expression (i.e., FasR or TRAIL-receptor 1) is all reported in SCLC.[29,30] Moreover, increased expression of c-FLIP, a non-cleavable homologue to caspase-8, was observed in SCLC.[31] In a patient with NSCLC, material consisting of approximately 100 specimens, somatic mutations of TRAIL receptor 2 were found in approximately 10% of the patients.[32] The TRAIL receptor 2 mutations reported were located in the death domain, a region required for appropriate apoptotic signaling.[32] However, if the mutations in TRAIL receptor 2 influenced the patient response to chemo- or radiotherapy or overall survival remains to be examined. Decreased expression of Apaf-1 was reported in NSCLC tumors compared with normal lung, whereas pro-caspase-9 and -3 were upregulated.[33] Enhanced expression of pro-caspase-3 was associated with poor prognosis in resected NSCLC,[34] whereas increased caspase-3 expression and/or activity was associated with increased survival in another study.[35] The localization of Apaf-1 to nucleus was also reported to predict survival in patients with early-stage NSCLC.[36]

With respect to Bcl-2 family proteins, we reported that radioresistant NSCLC cells display no or little Bak or Bax activation compared with radiosensitive NSCLCs or SCLCs.[37] The prognostic value of Bcl-2 for survival and/or chemo- or radiotherapy responses among patients with lung cancer was recently reviewed.[38] Increased Bcl-2 expression was found in certain NSCLC subtypes and was proposed to have slightly good prognostic value.[38] In the same report, Bax was found not to have any prognostic value, even for chemotherapy responses.[38] The expression and prognostic value of IAPs and HSPs in NSCLC has also been reviewed.[39] Briefly, c-IAP-1, XIAP, and survivin were reported to be differentially expressed in a panel of SCLC and NSCLC cell lines in a non-tumor type-dependent manner.[40] In contrast, c-IAP-2 was expressed at a significantly higher level in NSCLC lines compared with SCLC lines.[40] However, in a clinical LC material, c-IAP-1, -2, and XIAP were reported not to correlate to clinically related prognostic factors (e.g., tumor size, stage, histology, and grade) or to tumor chemotherapeutic response.[41] In contrast, a high survivin expression was reported to correlate to poor prognosis and local control after radiotherapy.[42] Analysis of HSP72 and HSP27 in human NSCLC and SCLC cell lines did not reveal any correlation to radiosensitivity in the cell line panel.[43] When examining HSP27 and HSP70 in NSCLC clinical specimens, expression was found in 60% of the cases, and HSP70 expression was correlated to histopathological differentiation, clinical stages, smoking history, or lymph node metastasis.[44]

It is well established that lung cancer, especially NSCLC, is driven by increased growth factor signaling. Thus IGF-1R, EGF-R (erbB1), or K-Ras are all often over-expressed or constitutively active in NSCLC and/or SCLC and may cause increased anti-apoptotic signaling.[45,46] Moreover, we have also shown that deficiency in activation of MAPKs such as JNK and/or p38 may also contribute to impaired radiation-induced apoptotic responses.[37] Impeded JNK activity may result from increased expression of the phosphatase MKP1/CL100 within NSCLC cells.[47]

APOPTOSIS-BASED THERAPIES

Several concepts of increasing apoptotic signaling as a way to improve chemo- and radiotherapy responses have been introduced and are in preclinical development to allow clinical use or have entered into clinical trials.[48,49] One way is to reactivate death receptor signaling; this strategy has been tested for therapeutic purposes.[50,51] TNFα was the first choice, and although it was capable of causing tumor cell kill, adverse toxic side effects abolished clinical applicability.[50] Instead, the TNFα-related apoptosis, inducing ligand TRAIL, which bind to TRAIL-R1 (DR4) and TRAIL-R2 (DR5/Killer), has shown promising results in the recombinant form alone or together with chemo- or radiotherapy.[50] Another approach that has been examined in several preclinical studies is to use agonistic TRAIL receptor monoclonal antibodies (mAbs).[51] Currently, phase I and II clinical trials with such mAbs directed against either DR4 (HGS-ETR1) or DR5 (HGS-ETR2) are ongoing for patients with NSCLC.[51] Using a tumor-specific gene delivery of the TRAIL gene to NSCLC cells in a xenograft mouse model, Chang et al.[52] recently showed a radiosensitizing effect involving induction of apoptotic signaling, inhibition of tumor growth, and prolonged survival of the tumor-bearing mice.

In preclinical lung cancer models, alleviation of IAP function using either antisense or siRNA or peptides mimicking the endogenous IAP inhibitor Smac has been tested either alone or in combination with chemo- or radiotherapy and have, to some extent, been promising.[14,53–55] Strategies in which Bcl-2/Bcl-xL expression is inhibited or BH3-mimetics applied have also been introduced. Thus, antisense against Bcl-2 (Oblimersen) has been tested in NSCLC and other tumor types and has reached phase III trials.[56,49] Mutations in the p53 gene, which impede its function as a transcriptional regulator of apoptosis, are common in both NSCLC and SCLC (50% and 70%, respectively).[57] Hence, one alternative to revert chemo- or radiotherapy resistance in lung cancer may therefore be to restore p53 function. This has been clinically tested in NSCLC by using wildtype-p53 gene transfer either alone or in combination with chemotherapy.[58] Preclinical development of peptides or small molecules that can reactivate mutant p53 is also ongoing.[49] However, the clinical usefulness of such an approach awaits further studies.

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