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New Drugs

Inhibitor of Apoptosis (IAP) proteins as therapeutic targets for radiosensitization of human cancers

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SUMMARY

Radiotherapy initiates a variety of signaling events in cancer cells that eventually lead to cell death in case the DNA damage cannot be repaired. However, the signal transduction pathways that mediate cell death in response to radiation-inflicted DNA damage are frequently disturbed in human cancers, contributing to radioresistance. For example, aberrant activation of antiapoptotic programs such as high expression of Inhibitor of Apoptosis (IAP) proteins has been shown to interfere with the efficacy of radiotherapy. Since IAP proteins have been linked to radioresistance in several malignancies, therapeutic targeting of IAP proteins may open new perspectives to overcome radioresistance. Therefore, molecular targeting of IAP proteins may provide novel opportunities to reactivate cell death pathways that mediate radiation-induced cytotoxicity. A number of strategies have been developed in recent years to antagonize IAP proteins for the treatment of cancers. Some of these approaches have already been translated into a clinical application. While IAP protein-targeting agents are currently being evaluated in early clinical trials alone or in combination with conventional chemotherapy, they have not yet been tested in combination with radiation therapy. Therefore, it is a timely subject to discuss the opportunities of antagonizing IAP proteins for radiosensitization. Preclinical studies demonstrating the potential of this concept in relevant *in vitro* and *in vivo* models underscore that this combination approach warrants further clinical investigation. Thus, combination protocols using IAP antagonists together with radiotherapy may pave the avenue to more effective radiation-based treatment options for cancer patients.

Introduction

The anticancer activity of most cytotoxic therapies which are currently used in the clinical management of cancer patients including radiotherapy is based on their ability to activate cell death programs such as apoptosis in cancer cells. Apoptosis or programmed cell death is an intrinsic program that is in place in every cell of the human body, including cancer cells.[1] A characteristic feature of human cancers is the inability to mount a proper apoptotic response during tumor progression or upon treatment with cytotoxic therapies.[2] Therefore, evasion of apoptosis constitutes a critical cause of primary or acquired treatment resistance that frequently occurs in various human cancers. This also applies to the resistance of cancers to radiotherapy, one of the main pillars of cancer therapy. Therefore, a better understanding of the molecular events that are responsible for the defects encountered in the apoptosis signaling network may offer novel perspectives to tackle treatment resistance, including radioresistance of human cancers. This review focuses on apoptosis resistance caused by aberrant expression and/or function of Inhibitor of Apoptosis (IAP) proteins, a family of antiapoptotic proteins, and discusses the opportunities of how the targeting of IAP proteins can be translated into the design of new radiation-based treatment protocols.

Signaling pathways in radiation-induced cell death

Radiation-induced signaling events can be initiated in distinct cellular compartments, for example the nucleus, the cytosol or the plasma membrane.[3] In response to DNA damage that is sensed in the nucleus, the tumor suppressor and checkpoint protein p53 accumulates and becomes activated, thereby initiating cell cycle arrest, DNA repair and, in case of severe DNA damage, the induction of cell death.[4] Furthermore, radiation can stimulate the production of reactive oxygen species (ROS) that can cause perturbation of the mitochondrial pathway of apoptosis.[5] In addition, ionizing radiation can damage the plasma membrane, leading to the generation of ROS which may cause lipid oxidative damage and production of bioactive molecules.[6] In addition, ROS production in response to irradiation has been associated with glutathione depletion and mitochondrial perturbation.[7] Radiation-induced DNA damage can also lead to the activation of stress-activated protein kinases, e.g.

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c-Jun NH2-terminal kinase (JNK).[8] All these cellular events in response to irradiation can eventually lead to the activation of a common effector phase of apoptosis, which is executed by a family of proteases called caspases.[9] Caspase activation can result in amplification of the death signal via the protease cascade, since caspases can activate each other via proteolytic cleavage.[10] In addition, feedback loops linking caspase activation to mitochondrial dysfunction contribute to amplification of cell death induction upon irradiation.[11]

Cell death by apoptosis can in principle proceed via two major routes, i.e. the extrinsic (death receptor) pathway and the intrinsic (mitochondrial) pathway.[12] In the extrinsic pathway, transmembrane death receptors of the tumor necrosis factor (TNF) receptor superfamily can trigger caspase activation and cell death upon binding of their cognate ligands.[13] The mitochondrial pathway of apoptosis is initiated by a variety of intracellular stimuli, including ROS and bioactive lipids, resulting in the release of proteins from the mitochondrial intermembrane space into the cytosol, for example cytochrome c and Smac.[14] Once in the cytosol, cytochrome c promotes caspase-3 activation, while Smac binds to and neutralizes IAP proteins, thereby releasing the breaks on caspase activation.

Inhibitor of Apoptosis (IAP) proteins are a family of endogenous antiapoptotic proteins.[15] All IAP proteins contain a baculoviral IAP repeat (BIR) domain which is the structural constituent for their classification as IAP proteins.[15] In addition to one to three of these BIR domains, IAP proteins can also contain other structural domains including the Really Interesting New Gene (RING) domain and the caspase-activating and recruitment domain (CARD).[15] The RING domain is an E3 ubiquitin ligase that is responsible for ubiquitination and proteasomal degradation of multiple substrates, including Smac and IAP proteins.[16]

Among the eight human IAP proteins, XIAP has been reported to exert the strongest antiapoptotic function,[17] which has been linked to its ability to bind to caspase-3,-7 and -9. In addition, ubiquitination and proteasomal degradation of proapoptotic factors, for example Smac and activated caspases, via the E3 ligase activity of XIAP can contribute to its antiapoptotic properties.[16] Also, XIAP has been implicated in the regulation of additional signaling pathways, including NF-κB activation.[18,19]cIAP1 and cIAP2 are involved in both canonical and non-canonical NF-κB signaling.[20] In the canonical NF-κB pathway, cIAP1 and cIAP2 are important mediators of non-degradative ubiquitination of the kinase RIP1, which is important for receptor-mediated NF-κB activation.[20] Furthermore, cIAP1 and cIAP2 restrain non-canonical NF-κB activation in unstimulated cells by triggering the constitutive degradation of NIK via the proteasome.[20] NIK is a key kinase that is involved in the initiation of the non-canonical NF-κB cascade.

Targeting IAP proteins for radiosensitization

On theoretical grounds, targeting IAP proteins may present a particularly promising approach to overcome radioresistance, since some IAP proteins such as XIAP and cIAP1 are among the proteins that can be regulated at the level of translation under cellular stress conditions.[21] Accordingly, the messenger RNA (mRNA) molecules of XIAP and cIAP1 are translated via an internal ribosome entry site (IRES).[22,23] This IRES site allows continued translation of the protein even under cellular stress conditions when CAP-dependent translation is usually blocked, for example following irradiation, endoplasmic reticulum (ER) stress, UV exposure or anoxia, and ensures that XIAP and cIAP1 protein expression levels are maintained.[21,24,25] Recently, MDM2 has been reported to be involved in the regulation of XIAP translation upon irradiation.[25] Irradiation-triggered DNA damage induced dephosphorylation of MDM2 and its cytoplasmic localization, thereby promoting IRES-dependent translation of XIAP.[25] Interestingly, MDM2 was shown to physically interact with the IRES of the 5′-UTR region of XIAP, and to stimulate XIAP IRES activity.[25] This ability to keep up IAP protein expression levels contributed to the ability of cancer cells, e.g. MDM2-overexpressing cells, to evade the induction of apoptosis following irradiation and thus conferred radioresistance.[25] Therefore, interfering with IAP protein expression or function may prove to be particularly suitable to restore sensitivity to radiation-induced apoptosis in cancer cells.

Against this background, a number of different strategies have been developed in recent years to antagonize aberrant IAP protein expression and/or functions in human cancers in order to overcome radioresistance and to increase radiosensitivity. In a first approach, genetic intervention studies tested the functional relevance of IAP proteins as targets for radiosensitization. Based on the evidence that XIAP exerts the most pronounced antiapoptotic functions among the eight human IAP proteins,[17] antisense oligonucleotides were designed to downregulate XIAP expression levels.[26] Furthermore, small molecule antagonists were developed that mimick the N-terminal part of Smac, which is the critical domain for the binding of Smac to XIAP as well as to other IAP proteins.[27,28] Structural studies identifying the binding pockets and interaction sites of XIAP and its endogenous antagonist Smac have greatly facilitated these structure-guided drug development efforts.[29]

Genetic interventions to target IAP proteins

To test the therapeutic potential of neutralizing IAP proteins to increase radiosensitivity of human cancers, a number of different genetic strategies have been employed (Table 1). For example, overexpression of Smac, the endogenous inhibitor of IAP proteins, has been reported to present a potent mean to increase radiation-induced cell death in a variety of human cancers, including neuroblastoma, glioblastoma, pancreatic and breast carcinoma.[30,31] Both the full-length and the mature forms of Smac were reported to significantly potentiate radiation-induced apoptosis and to reduce clonogenic survival.[30,31] Full-length Smac, which contains a mitochondrial translocation sequence, resides in the mitochondrial intermembrane space and is released from the mitochondria into the cytosol upon the induction of apoptosis. The mature version of Smac lacks the mitochondrial translocation sequence and is therefore constitutively expressed in the cytosol. Overexpression of either form of Smac increased γ-irradiation-induced activation of the caspase cascade, promoted mitochondrial outer membrane permeabilization and the release of cytochrome c from the mitochondria into the cytosol, leading to increased caspase-3 activation and caspase-dependent apoptosis.[30] Experiments showing that a broad-range caspase inhibitor or a relatively selective caspase-2 inhibitor blocked mitochondrial outer membrane permeabilization upon γ-irradiation of Smac-overexpressing cells suggested that Smac facilitates caspase activation upstream of mitochondria.[30] Similarly, overexpression of full-length Smac or the mature form of Smac was demonstrated in another study to promote irradiation-induced apoptosis in breast cancer cells.[31] Ectopic expression of Smac caused increased interaction of Smac with IAP proteins following irradiation, resulting in enhanced caspase-3 activation and apoptosis.[31] By comparison, overexpression of Smac did not affect the initial DNA damage and/or cellular stress response, as no differences in γH2AX or RAD51 foci formation, NF-κB activation, p53 accumulation or cell cycle arrest in response to γ-irradiation were observed in Smac-overexpressing cells compared to vector control cells.[30]

In addition to overexpression of Smac, knockdown of XIAP by RNA interference technology resulted in a significant increase in