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Mini-review

Radiotherapy and TRAIL for cancer therapy

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ABSTRACT

The use of radiotherapy and concomitant chemotherapy substantially improved cure rates in patients with different malignant tumours. However, it is unlikely that further improvements based on conventional chemotherapy may be achieved in the future since increased rates of acute side effects already limit the value of these approaches. Additionally, the increased local control rates are counterweighted by still high rates of distant failures resulting in low net gains for the patients. Thus, there is a currently unmet need for the integration of target-specific drugs improving local control as well distant control into radiation based treatment protocols. In this regard, the death-receptor ligand TNF-α-related apoptosis-inducing ligand (TRAIL/Apo2L) and TRAIL-receptor agonistic antibodies were shown to display a high selectivity for tumour cells and act synergistically with conventional chemotherapy drugs and radiation. Up to now it has been shown that radiation strongly sensitises malignant cells to TRAIL and TRAIL-agonistic antibodies. Synergistic induction of apoptosis was demonstrated in a majority of malignant cell types and xenograft models. Especially in those cells types displaying only weak responses to either treatment alone, strong sensitising effects were described. Moreover, in merely all normal cells and tissues no synergistic effects were found. Depending on cell type and experimental setting, the efficacy of combined treatment is determined by the p53-status, the balance between pro- and anti-apoptotic Bcl-2 proteins and modulation of TRAIL-receptor signal transduction.

1. Introduction

1.1. The role of apoptosis in carcinogenesis and cancer treatment

Apoptosis resistance is a central hallmark of carcinogenesis – consequently the efficacy of all anti-neoplastic treatments is limited by the presence of cells displaying alterations in their apoptotic machinery (for review see [1]). As selective induction of apoptosis in malignant cells may represent a central therapeutic strategy in radiation oncology, clinical efficacy is related at least partially to the inherent genetic and epigenetic changes of the death machinery in the tumour cells [2]. Elucidating the underlying mechanistic changes in malignant cells may thus trigger the development of new therapeutic approaches.

1.2. Intrinsic and extrinsic apoptotic pathways

Irrespective of the apoptotic stimulus applied, cell death-signalling ultimately culminates in a common downstream signal, namely the activation of cystein-aspartate-specific proteases, called caspases. Caspases in turn induce the degradation of central intracellular components like DNA, RNA and proteins through activation of RNAses, DNAses and proteases (for a review on receptor mediated apoptosis see [3]).

Ionising radiation and chemotherapeutic agents induce caspase activation mainly through changes in the balance of pro-versus antiapoptotic Bcl-2 proteins, thereby affecting mitochondrial integrity which leads to the release of

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pro-apoptotic substances, e.g. cytochrome *c*, from the mitochondrion and finally to caspase activation.

In contrast, death-receptors activate caspases through direct autoproteolytic cleavage at the intracellular death-receptor domain.

Although distinct in theory, both pathways are highly interconnected and are tightly regulated at different levels. In this context, the paradigm of type I – versus type II cells, originally described for CD95 triggered cell death [4], may serve as an example. In type I cells, activation of death-receptors is sufficient to induce the apoptotic phenotype, while in type II cells the amount of co-activation of the mitochondrial pathway sets the threshold for death-receptor induced effector-caspase activation [4]. An important mechanism connecting death-receptor signalling to the mitochondrion is mediated through formation of truncated Bid, which is formed through cleavage by caspase-8 [5–7]. Truncated Bid (t-bid) through inactivation of anti-apoptotic Bcl-2-family members and direct interaction with the pro-apoptotic molecule Bax [8] induces the release of pro-apoptotic factors from the mitochondrion [9].

1.3. Receptor mediated apoptosis

Receptor mediated apoptosis mainly occurs via cell surface receptors which belong the TNF-Superfamily (TNFR1, CD95/Apo-1/Fas, TRAIL-R1/DR4 and TRAIL-R2/DR5) and their respective ligands (TNF-α, CD95L/Apo1L/FasL, TNF-α-related apoptosis-inducing ligand: TRAIL/Apo2L). Although belonging to the same family of receptors there are several differences between TRAIL-, CD95- and TNF-death receptor signalling. In the following only TRAIL-receptor signalling will shortly be discussed.

Five TRAIL receptors can be distinguished: The proapoptotic receptors TRAIL-R1 (DR4) [10], TRAIL-R2 (DR5) [11,12] and the decoy receptors TRAIL-R3 (DcR1) [11,13] and TRAIL-R4 (DcR2) [14], the latter two lacking a functional intracellular death domain. Osteoprotegerin is similar in sequence to the receptors mentioned above. However, it does not bind TRAIL at physiological temperatures. Osteoprotegerin is implicated in the regulation of bone metabolism. Its function in death-receptor mediated apoptosis is not well understood. TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2) possibly exert their antiapoptotic action through competitive binding of TRAIL and hetero-multimerisation with DR5 [11,15,16].

DR4 and DR5 are the only proapoptotic TRAIL-receptors containing a functional intracellular death domain. Receptor activation by agonistic ligands results in receptor trimerisation and recruitment of FADD (Fas associated death domain) and pro-caspase-8 or pro-caspase-10, leading to the formation of the death-inducing signalling complex (DISC). Following the autoproteolytic activation of pro-caspase-8 or pro-caspase-10 [17] at the DISC, active caspase-8 or -10 in turn cleave the effector pro-caspase-3 which leads to the apoptotic phenotype.

This process is regulated on different levels. For example c-FLIP a catalytically inactive caspase-8/10 homologue is a negative regulator of caspase activation at the DISC (for review see [18]). IAPs (inhibitor of apoptosis proteins) are another class of negative regulators, which act through direct inhibition of caspases [19]. The action of IAPs is counteracted by Smac/DIABLO which is released from the mitochondria, after breakdown of the mitochondrial integrity.

The transcription factor NF-κB is another important candidate involved in the regulation of TRAIL-induced apoptosis. NF-κB was shown to inhibit the expression of DR4 and DR5 and caspase-8 by its RelA/p65 subunit. Furthermore, RelA/p63 positively regulates the expression of survivals factor cIAP1 and cIAP2 [20,21], and may also play a role in relative resistance of normal cells to TRAIL-induced apoptosis [22].

1.4. TRAIL as a potential therapeutic agent

The finding that TRAIL exerted a highly selective cytotoxicity towards malignant cells was the trigger to investigate the potential of TRAIL as an anti-neoplastic agent [23–25]. Currently is has been shown that recombinant TRAIL and monoclonal TRAIL-receptor agonistic antibodies possess a favourable safety-profile in a number of clinical phase I/II trials [26–28]. An overview of published and ongoing clinical studies can be found in a review by Ashkenazi [29] and in the chapter by Elisabeth de Vries in this issue.

Unfortunately, many cancer cells are resistant to TRAIL-induced apoptosis even at high doses [30], limiting the clinical use of TRAIL as a mono-therapeutic agent.

The following section will focus on the experimental results of combined treatment with ionising radiation and TRAIL-agonists *in vitro* and *in vivo*.

2. TRAIL-receptor based approaches in combination with ionising radiation

2.1. Rationale

In principle, curatively intended radiotherapy basically aims at eradicating all clonogenic cells within a tumour. In theory this could easily be achieved for every malignant tumour simply by escalating the radiation dose. However normal tissues surrounding a tumour limit the clinically useable radiation doses. Therefore, combined strategies have been introduced, finally achieving improved local cell kill rates without increasing the rate of late effects within the treated area.

In this context, the introduction of chemo-radiation protocols has been a key step. After initial experiments clearly documented synergistic cell death induction following combined treatment with cytotoxic drugs and ionising radiation [31], these findings were successfully translated into clinical reality. In this regard, the introduction of combined protocols increased local control and also survival rates in different cancer sides including head and neck cancer or cervical cancer by roughly 10–20 percent without an parallel increase in late tissue toxicity [32,33].

More recently, the addition of targeted drugs e.g. the EGFR-antagonist cetuximab to ionising radiation, was shown to also improve local control as well as overall survival in head and neck cancer patients [34].