An introduction to molecular targeted therapy of cancer

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ABSTRACT

The rapidly advancing elucidation of molecular targets in human cancers during the last decade has provided an excellent basis for the development of novel therapeutics. A huge variety of potential target structures have been identified, many of which are already being exploited for therapeutic purposes. This review introduces the reader into the concept of molecular targeted therapies, and provides some prototypic examples.

Key words: anti-cancer therapy, tyrosine kinase, apoptosis, epidermal growth factor (EGF), death receptors

INTRODUCTION

During the recent years, there has been an increasing and rapid development of molecular markers as targets for innovative therapeutic concepts (“Targeted Therapy”). A high number of molecules have become therapeutic targets already, especially growth factors and growth factor receptors, molecules of signal transduction, tumor-associated antigens, molecules of intracellular protein metabolism (proteasome inhibitors), factors regulating cell survival, cell cycle and cell death, and molecules associated with invasion, metastasis and angiogenesis. An overview on major examples for targets that already entered clinical trials is given in Fig. 1. Some of these molecular targeted compounds are not only efficient as tumor therapeutics, but also improve the patient’s quality of life by, for example, reducing pain associated with the reduction of bone which is true, e.g., for compounds as zoledronic acid [1-6]. The list of targets for molecular therapy is growing daily. In the following, we will select representative examples to illustrate the potential, but also open problems to solve of targeted therapy.

REVIEW

Example for success: targeting tyrosine kinase receptors, for example EGF-R

There has been an impressive development of compounds targeted against tyrosine kinase receptors [7]. Here, targeting concepts directed against c-erb-B2 (HER2), such as Herceptin especially in breast cancers, c-Kit-targeted therapy (Gleevec) in Bcr/Abl-positive leukemias and GIST-tumors, VEGF/VEGF-R-targeted compounds [8,9], and a number of therapeutic concepts targeting EGF-receptor (EGF-R), are standing out as major examples that already have led, or will most probably lead, to paradigm shifts in the treatment of major tumor diseases. With increasing numbers of clinical studies, a part of them having been accompanied by molecular translational studies, however, it becomes clear that most certainly the therapeutic response towards suchlike compounds to a considerable extent will be defined by the individual molecular conditions of the individual patient, the genetic- or population-based background of a patient, and either acquired or inherited peculiar characteristics and changes within the gene encoding the target, such as amplifications, mutations, or polymorphisms.

This can be illustrated, for example, by first experiences from clinical studies on compounds targeting the EGF-receptor. The EGF-receptor is being overexpressed in a
number of solid carcinomas, such as colorectal or certain types of lung cancers [10], and its prognostic impact has already been shown for these tumor entities for certain patient subgroups [10-12]. Binding of the ligand EGF leads to a dimerization either with another EGF-R-molecule, or with a molecule out of the Erb-B-receptor tyrosine-kinase family. This is followed by the phosphorylation of the intracellular domain, activating a number of, for example, Ras-associated signalling cascades that can initiate phenomena such as tumor cell proliferation, invasion, metastasis, or anti-apoptosis [5,6]. During the recent years, diverse therapeutic strategies targeting EGF-R have been developed. Small molecular compounds targeting EGF-R are directed against the tyrosine kinase domain of EGF-R and inhibit its activation, thereby inhibiting EGF-R initiated signalling [13-15]. Other EGF-R-targeted strategies are based on antibodies [16-24], e.g., Cetuximab [25,26]. A number of studies already have been conducted especially concerning the tyrosine-kinase inhibitors in colorectal [10] and also lung cancer [27-30]. Especially, large studies in non-small cell lung cancer such as the ISEL- or BR21-study on 1692 or 731 patients have shown that the best survival and best response to therapy was observed in patient subgroups with Asian population background, female gender, adenocarcinoma, and no history of smoking. Furthermore, in different studies, the level of EGF-R protein expression or amplification of the EGF-R gene was associated with response to EGF-R-based therapy [31-38]. Certain studies show an association of certain mutations within the EGF-R gene with the clinical response towards small molecular EGF-R targeted compounds [33]. Most of these mutations have been found within exons 18 to 21 within the EGF-R-gene [32]. In addition, it has been shown that certain mutations within the EGF-R-gene can be associated with the development of a secondary resistance to therapy [32,33]. In addition, patients harbouring activating k-ras-mutations in non-small cell lung cancers most often show resistance towards EGF-R-based tyrosine kinase inhibitors [32]. In such cases, a combination with, for example-Ras-targeted compounds may be necessary for an individual patient. For antibodies such as Cetuximab®, preliminary data suggest that response might be independent of EGF-R-mutations, in contrast to the tyrosine kinase inhibitors. Therefore, an antibody-based therapy in case of secondary mutations could be an option. In general, an antibody-based therapy directed against EGF-R (Cetuximab) is already accepted in colorectal cancer for patients with metastasis, especially in case of an irinotecan resistance [25,26]. At present, ongoing phase III-studies are investigating the potential of Cetuximab in the situation of lung cancer (e.g. Manegold C et al., the ongoing Gemtax IV-Study), and the FLEX-Study recently has shown superiority of Cetuximab with chemotherapy as compared to chemotherapy alone in first-line treatment of NSCLC (ASCO 2008). In parallel to these ongoing studies, first molecular translational research results, in part of them by our own group, implicate first potential molecular indicators of therapy response, and also implicate that this antibody is able to inhibit different steps of metastatic cascade [36]. Taken together, the initial results of the particular example of EGF-R-targeted therapy illustrate that particular molecular, and also potentially genetic, conditions can modify and affect the response to targeted therapy concepts. It emphasizes the notion that detailed molecular analysis of the individual tumor in the individual patient need to accompany further studies
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**Figure 2. Apoptosis pathways.**

Apoptosis pathways can be initiated by ligation of death receptors (DR) such as CD95 or TRAIL receptors (TRAIL-Rs) by their respective ligands, e.g. CD95 ligand (CD95L) or TRAIL, followed by receptor trimerization, recruitment of adaptor molecules (FADD) and activation of caspase-8 (receptor pathway). The mitochondrial pathway is initiated by the release of apoptogenic factors such as cytochrome c, Smac or AIF from mitochondria in the cytosol. Apoptosis can be inhibited by Bcl-2 or by “Inhibitor of Apoptosis Proteins” (IAPs). Smac promotes apoptosis by neutralizing IAP-mediated inhibition of caspase-3 and -9. See text for more details.

**Example: targeting apoptosis pathways for cancer therapy**

Moreover, a number of strategies have been developed that target the apoptotic machinery in cancer cells. Apoptosis or programmed cell death is the cell’s intrinsic death program that plays an important role in various physiological and pathological situations and is highly conserved throughout evolution [39]. Tissue homeostasis is maintained by a subtle balance between proliferation on one side and cell death on the other side [40]. As a consequence, too little apoptosis can contribute to tumor formation, progression and treatment resistance [41]. Moreover, one of the most important advances in cancer research in recent years is the recognition that killing of tumor cells by anticancer therapies commonly used in the treatment of human cancer, e.g. chemotherapy, γ-irradiation, immunotherapy or suicide gene therapy, is predominantly mediated by initiating programmed cell death, i.e. apoptosis, in cancer cells [42,43]. The elucidation of signaling pathways involved in the regulation of apoptosis in cancer cells over the last decade has led to the identification of key apoptosis regulatory molecules that may serve as molecular targets for cancer therapy. In principle, apoptosis-based cancer therapeutics may aim at directly activating apoptosis pathways in cancer cells, at restoring defects in the apoptotic machinery or at disabling the antiapoptotic function of molecules involved in treatment resistance. Such strategies may open new perspectives to overcome apoptosis resistance in a variety of human cancers. Some examples how apoptosis pathways could be targeted for cancer therapy will be discussed in the following sections.

**APOPTOSIS SIGNALING PATHWAYS**

There are two principle pathways of apoptosis, the receptor or extrinsic and the mitochondrial or intrinsic pathway (Fig. 2) [43]. Stimulation of either pathway eventually fuels into activation of caspases, a family of cysteine proteases that act
as common effector molecules in various forms of cell death [44]. Caspases are synthesized as inactive proenzymes. Once activated, they cleave various substrates in the cytoplasm or nucleus causing characteristic morphological features of apoptotic cell death [44]. In the extrinsic apoptosis pathway, stimulation of death receptors of the tumor necrosis factor (TNF) receptor superfamily, e.g. CD95 (APO-1/Fas) or TRAIL receptors, results in activation of the initiator caspase-8, which in turn can directly cleave downstream effector caspases such as caspase-3 [45]. Also, activation of caspase-8 may link the receptor to the mitochondrial pathway by cleaving Bid, a Bcl-2 family protein with a BH3 domain only that translocates to mitochondria upon cleavage to initiate a mitochondrial amplification loop [46]. In the mitochondrial pathway, the release of apoptogenic factors such as cytochrome c, apoptosis inducing factor (AIF), second mitochondria-derived activator of caspase (Smac)/direct IAP Binding protein with Low PI (DIABLO) or Omi/high temperature requirement protein A (HtrA2) from the mitochondrial intermembrane space into the cytosol initiates caspase-3 activation [47]. Cytochrome c promotes caspase-3 activation through formation of the cytochrome c/Apaf-1/caspase-9-containing apoptosome complex, while Smac/DIABLO promote caspase activation through neutralizing the inhibitory effects of inhibitor of apoptosis proteins (IAPs) [47]. Because of the potential detrimental effects on cell survival in case of inappropriate caspase activation, activation of caspases has to be tightly controlled. The anti-apoptotic mechanisms regulating cell death have also been implicated in conferring drug resistance to tumor cells.

APOPTOTIC SIGNALING MOLECULES AS TARGETS FOR CANCER THERAPY

Most anticancer therapies primarily act by inducing apoptosis in cancer cells [48]. Accordingly, defects in apoptosis programs may lead to resistance of cancers to current treatment approaches. Since evasion of apoptosis is a characteristic feature of human cancers, strategies designed to restore defective apoptosis programs in cancer cells may overcome intrinsic or acquired resistance of tumor cells to current regimens [49]. Also, apoptosis targeted therapies may increase the responsive rate of tumors towards conventional treatments that are currently used in the clinic, e.g. chemo- or radiotherapy [43].

Targeting death receptors for cancer therapy

The idea to trigger death receptors in order to induce apoptosis in cancer cells is attractive for cancer therapy, since death receptors are directly linked to the cell’s intrinsic death machinery [50]. Death receptors are cell surface receptors that belong to the tumor necrosis factor (TNF) receptor gene superfamily [45,50,51]. These receptors exert a wide range of biological functions in addition to signal to cell death. For example, death receptors have also been implicated in the regulation of survival, differentiation and immune responses [45,50,51]. Death receptors share an intracellular domain called „death domain“, which transmits the death signal from the cell’s surface to intracellular signaling pathways. The best-characterized death receptors include CD95 (APO-1/Fas), TNF receptor 1 (TNFRI), TNF-related apoptosis inducing ligand (TRAIL) receptor 1 (TRAIL-R1) and TRAIL-R2. There exists also a family of corresponding ligands of the TNF superfamily that comprises CD95 ligand, TNFα or TRAIL. Binding of death receptors by their cognate ligands or by agonistic antibodies leads to oligomerization and activation of death receptors.

The death receptor ligand TRAIL is considered as a promising candidate for clinical development, since TRAIL preferentially kills cancer cells [52]. Recombinant soluble TRAIL or monoclonal antibodies targeting TRAIL receptors TRAIL-R1 or TRAIL-R2 were reported to induce apoptosis in a wide range of cancer cell lines and also in vivo in several xenograft models of human cancers [52-54]. Interestingly, TRAIL-R2 antibody-based therapy was recently reported as an efficient strategy not only to eliminate TRAIL-sensitive tumor cells, but also to induce tumor-specific T cell memory that afforded long-term protection from tumor recurrence [55]. Since a large proportion of human cancer turned out to be partially or completely resistant towards monotherapy with TRAIL despite the expression of both agonistic TRAIL receptors, TRAIL-based combination therapies were developed. To this end, TRAIL was reported to synergistically interact with chemotherapy or γ-irradiation in a variety of cancers [56,57].

Targeting the mitochondrial pathway for cancer therapy

Another approach to target apoptosis pathways for cancer therapy is to antagonize antiapoptotic Bcl-2 family members. The Bcl-2 family of proteins consists of both antiapoptotic members, e.g. Bcl-2, Bcl-XL, Mcl-1, as well as proapoptotic molecules [46]. The later comprise on one side multidomain proteins such as Bax, Bak and Bad and on the other side BH3-domain only molecules, e.g. Bim, Bid, Bmf, Noxa or Puma [46]. Bcl-2 family proteins play an important role in the regulation of the mitochondrial pathway of apoptosis, since they are involved in the control of mitochondrial outer membrane permeabilization [46]. There are currently two models how BH3-only proteins activate Bax and Bak during the course of apoptosis. According to the direct activation model [58], putative activators such as Bim and cleaved Bid (tBid) bind directly to Bax and Bak to trigger their activation, while BH3-only proteins that act as sensitizers, e.g. Bad, bind to the pro-survival Bcl-2 proteins. By comparison, the indirect activation model holds that BH3-only proteins activate Bax and Bak by binding and thus inactivating the various antiapoptotic Bcl-2 proteins that in turn inhibit Bax and Bak [59]. Imbalances in the ratio of anti- versus pro-apoptotic Bcl-2 proteins may tipp the balance towards tumor cell survival and thus may contribute to tumor formation and progression. Since high expression
of anti-apoptotic Bcl-2 family proteins may confer resistance to chemo- or radiotherapy by blocking the mitochondrial pathway of apoptosis, there has been much interest to develop strategies to overcome the cytoprotective effect of Bcl-2 and related molecules. A prominent example of these efforts is the development of the small molecule antagonist ABT-737, which binds to the surface groove of Bcl-2, Bcl-XL and Bcl-w that normally interacts with the BH3 domain of Bax or Bak [60]. By preventing the binding of antiapoptotic Bcl-2 proteins to Bax or Bak, ABT-737 frees Bax and Bak to oligomerize and to form pores in the outer mitochondrial membrane, promoting the release of cytochrome c from mitochondria into the cytosol. Studies in cancer cell lines and preclinical models demonstrate that ABT-737 as single agent can trigger apoptosis in some susceptible cancer types, e.g. those that critically depend on Bcl-2 for survival [60]. In addition, ABT-737 sensitizes cancer cells for apoptosis when combined with conventional chemotherapeutics [61]. Since ABT-737 targets Bcl-2/Bcl-xL but not Mcl-1, high expression of Mcl-1 may confer resistance to this novel agent. Indeed, several recent reports indicate that Mcl-1 represents a key determinant of ABT-737 sensitivity and resistance in cancer cells [62,63]. Collectively, these findings suggest that small molecule inhibitors of antiapoptotic Bcl-2 family proteins may open new perspectives to reactivate the mitochondrial pathway of apoptosis in cancer cells.

**Targeting “Inhibitor of Apoptosis Proteins” (IAPs) for cancer therapy**

Another promising therapeutic strategy directed at apoptosis regulators is the neutralization of “Inhibitor of Apoptosis Proteins” (IAPs). The family of endogenous caspase inhibitors “Inhibitor of Apoptosis Proteins” (IAPs) comprise eight human analogues, i.e. XIAP, c-IAP1, c-IAP2, survivin, apollon, livin/melanoma-IAP (ML-IAP), NAIP and ILP-2 [64]. IAPs have been reported to directly inhibit active caspase-3 and –7 and to block caspase-9 activation [64]. The role of survivin in the regulation of apoptosis and proliferation is more complex compared to other IAP family proteins, since in addition to regulation of apoptosis, survivin is involved in regulation of mitosis [65]. There is mounting evidence that cancer cells have an intrinsic drive to apoptosis that is held in check by IAPs. To this end, high basal levels of caspase-3 and caspase-8 activities and active caspase-3 fragments in the absence of apoptosis were detected in various tumor cell lines and cancer tissues, but not in normal cells [66]. Tumor cells in contrast to normal cells also expressed high levels of IAPs suggesting that upregulated IAP expression counteracts the high basal caspase activity selectively in tumor cells [66].

Since IAPs are expressed at high levels in the majority of human cancers, they present an attractive molecular target. Consequently, several strategies have been developed to target enhanced expression of IAPs in human malignancies. For the design of therapeutic small molecules directed against X-linked inhibitor of apoptosis protein (XIAP), the binding groove of the BIR3 domain of XIAP, to which Smac binds to after its release from mitochondria, has attracted most attention [67]. Smac peptides that neutralize XIAP through binding to its BIR2 and BIR3 domains were able to promote caspase activation and enhanced TRAIL- or chemotherapy-induced apoptosis. In addition, Smac peptides even substantially increased the antitumor activity of TRAIL in vivo in an intracranial malignant glioma xenograft model, resulting in complete eradication of established tumors [68]. Also, XIAP antisense oligonucleotides exhibited potent antitumor activity as single agent and in combination with clinically relevant chemotherapeutic drugs [69,70]. Recently, IAP antagonists were reported to kill cancer cells by inducing autoubiquitination of c-IAPs, NF-κB activation, and TNFalpha-dependent apoptosis [71-73]. Currently, XIAP antisense oligonucleotides are evaluated in phase I/II clinical trials either as single agent or in combination with chemotherapy in advanced tumors. Thus, Smac agonists, low molecular weight XIAP antagonists or XIAP antisense oligonucleotides are promising new approaches to either directly engage apoptosis or to lower the threshold for apoptosis induction in cancer cells.

**The challenge of today: defining the right patients for the right therapeutic concept**

The examples given above illustrate the high and promising potential of molecular targeted therapy. However, they also illustrate the increasing importance of including molecular diagnosis to achieve an appropriate patient selection for therapy. An increasing attention is begin given to the field of pharmacogenomics, which investigates the genetic conditions of patients defining a particular type of response to certain therapeutics [71]. For example, there is increasing evidence that genetic polymorphisms which, under normal conditions, are not relevant for a disease or a phenotype, can significantly modify the response to certain types of therapies, for example cytochrome p450-dependent substances [74]. Such polymorphisms can also influence the response not only to novel molecular targeted therapies, but also classical chemo- or radiation therapy. Prominent examples for this notion are certain enzymes involved in DNA-repair mechanisms. For example, certain polymorphisms within the XRCC3-gene (X-ray repair cross complementing group 3) have been shown to be associated with a significantly longer survival following Cisplatinum/Gemcitabine-based therapy in non-small cell lung cancer, as compared to Cisplatinum/Docetaxel-based therapy. The survival benefit resulting from these polymorphisms was observed especially in young patients with non-small cell lung cancer [75]. The consequence out of such a study would be that younger patients with non-small cell lung cancer harbouring particular polymorphisms of the XRCC3-gene would be treated with Cisplatinum/Gemcitabine rather than Cisplatinum/Docetaxel. In another study [76], it was shown that a particular polymorphism of the ERCC1-gene (excision repair cross complementing group 1), ERCC1-8092A/A, defines a particularly poor survival following treatment with Cisplatinum/Docetaxel. ERCC1 is an important enzyme
conducting nucleotide-excision DNA-repair that is known to remove DNA-adducts following Cisplatinum-based therapy. Certain ERCC1-polymorphisms affect ERCC1-expression, and it has been shown that NSCLC-patients with low ERCC1-expression respond better to Cisplatinum-based therapy than patients with high ERCC1 [77].

These are only two out of many recent examples illustrating that genetic polymorphisms within DNA-repair relevant for metabolizing DNA-changes following particular types of chemotherapy can significantly modify the therapeutic response of tumor patients towards classical therapy concepts. They illustrate that pharmacogenomics will be of increasing importance for optimizing therapeutic compounds towards the individual genetic and molecular conditions of an individual tumor patient in the future. Certainly, novel generations of targeted therapy strategies also will increasingly have to consider particular molecular or genetic variations and changes within patients for a further significant improvement of therapy response and survival of cancer patients. Therefore, individual genetic or inherited conditions that by themselves might not be causative for a disease, will become increasingly important even for sporadic types of cancers, and for the therapy of tumors with a non-familiar background.

CONCLUSION

Over the last two decades, the elucidation of molecular conditions, among them being signal transduction pathways involved in the regulation of tumor growth, cell death in human cancers, or molecular markers of cancer progression, have provided the fundamental basis for the development of molecular targeted therapies. Since such strategies are specifically directed against key components that are crucial for the cancer cell’s survival and function, they may be more selective and effective in killing malignant over non-malignant cells. While several approaches have already been translated into medical application, many concepts have still to be evaluated in (pre)clinical trials. Another main goal ahead with molecular targeted therapies will be considering the appropriate patient selection to enrich for a responsive population. Eventually, these efforts are expected to yield more effective yet less toxic treatment options for the sake of patients suffering from cancer.

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