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Targets for cancer therapy in childhood sarcomas

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SUMMARY

Development of chemotherapeutic treatment modalities resulted in a dramatic increase in the survival of children with many types of cancer. Still, in case of some pediatric cancer entities including rhabdomyosarcoma, osteosarcoma and Ewing's sarcoma, survival of patients remains dismal and novel treatment approaches are urgently needed. Therefore, based on the concept of targeted therapy, numerous potential targets for the treatment of these cancers have been evaluated pre-clinically or in some cases even clinically during the last decade. This review gives an overview over many different potential therapeutic targets for treatment of these childhood sarcomas, including receptor tyrosine kinases, intracellular signaling molecules, cell cycle and apoptosis regulators, proteasome, hsp90, histone deacetylases, angiogenesis regulators and sarcoma specific fusion proteins. The large number of potential therapeutic targets suggests that improved comparability of pre-clinical models might be necessary to prioritize the most effective ones for future clinical trials.

Introduction

About 12,000 children under 20 years of age are diagnosed each year with cancer in the US alone. Fifty years ago, such a diagnosis was a sentence of death for most patients due to the lack of effective therapies at that time and despite surgical resection of the tumor. This changed dramatically with the discovery of different cytotoxic chemotherapeutic agents able to kill proliferating cells which resulted in the development of effective therapies for many of these cancer types. Intensification of treatment and optimized treatment modalities, together with more effective supportive care to fight side effects, resulted in a continuous improvement of outcome in the second part of the last century. In the period 1996–2002 the overall 5-year survival rate has reached 79% [1]. For many of the pediatric cancer entities including Hodgkin lymphoma, retinoblastoma, Wilms tumor, and germ cell tumors, the survival rate nowadays even exceeds 90%.

However, for some other childhood cancer entities, chemotherapy still remains largely non-effective. Resistance to the drugs and metastatic spread represent the two most important mechanisms for therapy failure. Tumors belonging to this group include different types of sarcomas such as rhabdomyosarcoma, Ewing's sarcoma or osteosarcoma which reach an overall 5-year survival rate of 60–65% [2]. Specific subgroups of these tumors with a tendency of early metastasis such as alveolar rhabdomyosarcoma even have a much poorer prognosis. In addition, intensive chemotherapeutic treatment can result in a variety of long-term sequelae in childhood patients, including impairment of growth and development, a variety of organ dysfunctions and subsequent secondary malignancies, preventing further intensification of therapy with these drugs [3]. Therefore, based on the plateau which has now been reached with current treatment options there is an urgent need for alternative, more targeted treatment approaches.

This review will summarize some of the numerous recent developments in therapeutic approaches against childhood sarcomas with a special focus on rhabdomyosarcoma, Ewing's sarcoma and osteosarcoma.

Targeted therapy

To overcome problems associated with the unspecificity of the current therapeutic approaches, the concept of "targeted therapy" has been developed. Per definition, such an approach is based on the application of drugs (more) specifically targeting tumor cells and sparing normal cells. Two general approaches can be distinguished:

The first approach is based on differences in the physical presence of molecular markers between cancer and normal cells. Cancer-specific markers could serve as targets for antibodies or antibody-like molecules linked to cytotoxic agents which, upon binding, are able to kill these cells. Furthermore, immunological techniques may allow priming of specialized immune cells to recognize cancer-specific structures.

The second approach is based on differences in the dependency on certain functional characteristics between cancer and normal

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cells, which could be therapeutically exploited. Four different dependencies have been defined [4]. First, most cancer cells are dependent on the constant activity of only some of the underlying abnormalities ("oncogene addiction" or genetic dependence) (see also [5]). Second, in some cancer cells mutation of one pathway renders these cells dependent on another pathway (synergy dependence). Third, cancer cells are often dependent on the same signaling pathways as their normal counterparts (lineage dependence). Fourth, single tumor cells or the tumor as a whole can depend on activities coming from the host environment such as angiogenesis or growth factors produced by stromal cells (host dependence). Numerous studies demonstrated that inhibition of activities involved in these dependencies can either inhibit cell proliferation or induce apoptosis in tumor cells but leaves normal cells more or less unaffected, suggesting that cancer cells are to a much greater extent dependent on these functions than normal cells. Therefore, all these dependencies represent potential targets for a cancer cell specific treatment.

Based on these concepts, identification of dependencies for each tumor entity or maybe even each patient is a crucial prerequisite for the development of future targeted therapies. The process of defining novel targets includes several crucial steps such as target identification based on experimental data including gene expression or mutational analysis. However, the main work should be a careful pre-clinical validation both *in vitro* with clear effects on cell viability and tumor growth and *in vivo* using different animal models.

Technological advances during the past decade in the area of genome and transcriptome analysis, including sophisticated gene expression analysis as well as high throughput sequencing methods, accelerated identification of potential targets substantially and resulted in a plethora of potential specific targets in all kinds of cancer entities. Also in childhood sarcoma many potential targets have been defined and some of them have been pre-clinically evaluated. A comprehensive list of potential molecular targets for treatment of childhood sarcoma is given in Table 1 and will be discussed in detail in the following paragraphs.

Table 1
Therapeutic targets in rhabdomyosarcoma, Ewing's sarcoma and osteosarcoma.

Groups of therapeutic targets	Tumor type		
	Rhabdomyosarcoma	Ewing's sarcoma	Osteosarcoma
Receptors/growth factors	**IGF1R** [14,15,18–21] **c-Met** [31] **PDGFR** [45,47] **c-Kit** [47] CTGF/CCN2 [173] Midkine [174] CTLA-4 [190]	**IGF1R** [11–13,16,17,19–20] **c-Kit** [36,43–44,47–48] **PDGFR** [44,47,48] CD99 [184] IFN-α, IFN-β [185]	**IGF1R** [18–20] **c-Met** [25] **PDGFR** [34,41,47,48] Midkine [187] Notch [188] MUC18 [189] CTLA-4[190] Hedgehog [191]
Intracellular signaling molecules	**mTor** [57–59] PDK-1/AKT [175] Src [176] Mek/Erk [177,178] Estrogen receptor [179] Mirk/Dyrk1B [180] Smad4 [181] Retinoic acid [182]	**mTOR** [58] Src [176] Lyn [186]	**mTor** [55,58] c-jun [192,193] Src [176,198] Stathmin [194,195] α-CaMKII [196]
Cell cycle	**CDK4/CDK6** [82]	**CDKs** [78–81]	**CDKs** [78]
Apoptosis	**p53** [86,88] **Bcl-2** [89] **TRAIL** [93,102–105] Survivin [183]	**p53** [87] **Bcl-2** [89] **TRAIL** [94–97,101]	**p53** [87] **Bcl-2** [89] **TRAIL** [98–100,106] Mdm2, p21[197]
Proteasome	[108,112]	[109,112]	[110–112]
Hsp90	[115,116]	[116,117]	[118]
Histone Deacetylases	[125,128]	[122–125,127,131–132]	[125–127,129–130,132]
Angiogenesis	**VEGF** [144] **VEGFR** [140]	**VEGF** [137] **VEGFR** [140]	**VEGF** [142] **VEGFR** [140]
Cancer-specific fusion proteins	**PAX3/FKHR** [160–164]	**EWS/FLI1** [160,161]	

Targets discussed in the text are written in bold.

Receptor tyrosine kinases

Transmembrane receptor tyrosine kinases (RTKs) are important upstream elements of signaling cascades which regulate cell growth, proliferation and survival. Many different RTKs are implicated in tumorigenesis of numerous cancer types [6]. Localization at the cell surface makes them accessible not only for small molecule inhibitors, but also for inhibitory antibodies, highlighting them as key targets for cancer treatment. Importantly, in normal tissue activity of most RTKs is only mandatory during embryonic development and not essential during adulthood [7]. A series of RTKs has been linked to the development of childhood sarcomas including IGF1R, PDGFR, c-met and c-kit [8]. These RTKs have subsequently also been investigated as targets for therapy of these tumor types.

The IGF1R is one of the prototype targets in childhood sarcoma and has been extensively studied. Although no activating mutations are known for this RTK, a plethora of studies have linked aberrant activity of this kinase to different cancers (for a general review see [9], for a sarcoma specific review see [10]). In sarcomas, overexpression of the IGF1R itself, deregulated expression of ligands/ligand binding proteins or constitutive activation of downstream effectors might play a role. Different studies have evaluated the effect of IGF1R inhibition on growth of childhood sarcomas cell lines and xenografts, both with inhibitory antibodies and small molecule inhibitors [11–21]. In these studies, blockade of IGF1R activity has been shown to affect cell proliferation, survival and anchorage-independent growth *in vitro* and tumorigenesis, tumor invasion and metastasis *in vivo*. Since it also sensitizes cancer cells to chemo- and radiotherapy *in vivo*, IGF1R is one of the prime potential targets for treatment of these sarcomas. Indeed, the pre-clinical data initiated several clinical phase II trials which are currently ongoing and will evaluate the efficacy of IGF1R-targeting for pediatric sarcoma (Table 2).

The significance of the other three RTKs mentioned as therapeutic targets is less well documented. Numerous studies reported c-met expression in rhabdomyosarcoma and osteosarcoma [22–24] and c-met activity has been linked to regulation of proliferation, metastasis and resistance to chemotherapy in these tumors [25–29]. Furthermore, overexpression of HGF, the ligand for c-met, in an Ink4 null background leads to the development of RMS in mice [30]. In contrast, only few studies evaluated c-met as target for therapy. SiRNA-based silencing of c-met was found to inhibit proliferation, invasiveness, and anchorage-independent growth *in vitro*, and reduce tumor mass in a xenograft model of rhabdomyosarcoma [31]. In a second study, the c-met inhibitor K252a was able to revert HGF dependent growth of osteosarcoma cell lines [25]. However, based on promising findings obtained in numerous pre-clinical studies with c-met inhibitors for treatment of adult tumors [32], further evaluation of c-met in childhood sarcomas might be indicated.

PDGFR is expressed in all three types of childhood sarcomas discussed here [33–35] and c-kit expression has been found in Ewing's sarcoma and osteosarcoma [36–38]. Mutations were only found in the case of c-kit and in a small fraction of Ewing's sarco-