HER Tyrosine Kinases in Rhabdomyosarcoma

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EGFR is highly expressed by embryonal rhabdomyosarcoma (ERMS) tumors and cell lines, in some cases contributing to tumor growth. If not mutated, HER2 is not directly involved in control of RMS cell growth but can be expressed at significant levels, with a slight prevalence for alveolar RMS (ARMS). A minority of ERMS carries a HER2 mutation with driving activity on tumor growth. HER3 is frequently overexpressed by RMS and can play a role in the residual myogenic differentiation ability and in resistance to signaling-directed therapy. HER family members could be exploited for therapeutic approaches in two ways: blocking with antibodies or inhibitors the HER member driving tumor growth and targeting expressed HER members to vehiculate toxins or immune effectors.

rhabdomyosarcoma HER2 EGFR targeted therapy CAR-T precision medicine

1. Introduction

Rhabdomyosarcomas (RMS) are tumors of the skeletal muscle lineage, mainly occurring in children and adolescents. RMS are identified by the expression of markers of myogenic differentiation such as desmin, myogenin and MyoD1. The classification of RMS was initially based on pathological features; the two prevalent subtypes showed alveolar or embryonal-like pattern morphologies and are referred to as alveolar or embryonal RMS (ARMS or ERMS, respectively). Heterogeneity in histology, biomarkers, molecular driver events and clinical outcome led to a reclassification of RMS subtypes [1][2][3][4] that takes into account the molecular events. In fact, the study of gene expression profiles highlighted a major role for the presence/absence of a translocation between PAX3/7 and FOXO1 genes, leading to a major dichotomy between fusion-positive RMS and fusion-negative RMS [1][5]. A large genomic characterization was designed to further refine risk stratification based on additional molecular alterations [6].

The main oncogenic driver of fusion-positive RMS is the product of fusion itself (the PAX3/7–FOXO1 chimeric transcription factor), but a cooperating event is needed, such as gene amplifications (MYCN, CDK4 or MIR-17–92) or deletions (CDKN2A, loss of heterozygosity in 11p15.5) [7]. Mutations in BCOR, NF1, TP53 and PIK3CA were sporadically found in fusion-positive RMS [6]. PAX3/7–FOXO1 targets include receptor tyrosine kinases (RTK), which are consequently overexpressed and actively signaling along the RAS/Phosphatidylinositol 3-kinase (PI3K) axis [8].

Fusion-negative RMS have a higher degree of aneuploidy and a heterogeneous mutation burden comprising of coexisting and alternative mutations in RTK/RAS/PI3K pathway. Activation of the RAS pathway can be found

frequently and can be caused by mutations of RAS itself or by alternative events, either upstream RAS (such as mutations in RTK) or downstream RAS (such as BRAF) [7][9]. More than half of fusion-negative RMS showed mutation of any RAS pathway member [6]. Additional relevant mutations in BCOR, NF1 and TP53 were found in fusion-negative RMS [6]. A major hallmark of fusion-negative RMS is the loss of heterozygosity at 11p15 or uniparental paternal disomy of the entire chromosome 11 [9]. The 11p15 region contains various genes, including HRAS and insulin-like growth factor 2 (IGF2). IGF2 is consequently overexpressed due to the loss of imprinting. Loss of heterozygosity at 11p15 and activation of RAS pathway are early events in the genesis of fusion-negative RMS [10].

For high and very high-risk RMS new therapeutic strategies are urgently needed [11]. New therapeutic targets can be identified through the study of oncogenic drivers causing RMS. RTK of the HER family can be involved in the onset of RMS and in therapeutic targeting strategies.

2. Expression of HER Family Members in Human RMS Subtypes

High expression level EGFR was found in the majority of ERMS (Table 1), at quite strong expression levels, suggesting EGFR as an additional diagnostic marker for ERMS [3][12]. EGFR was only sporadically expressed by ARMS. The higher EGFR expression by ERMS versus ARMS was also observed at the mRNA level [13]. HER2 was expressed by a fraction of RMS, with a slight prevalence for ARMS [14]. Both EGFR and HER2 were phosphorylated in RMS, while normal skeletal muscle did not show any phosphorylated form of these RTK [15].

Table 1. The expression of HER family members in RMS subtypes.

HER Family Member	ARMS	ERMS	Other Subtypes	Intensity at Immuno-Histochemistry	Amplification/Mutation	Reference
EGFR	16%	76%		Moderate to strong	No amplification [a] at 7p11.2	[14]
	13%	84%	42% [b]	Strong in ERMS		[16]
	32%	55%	73% [c]			[17]
	29%	93%				[12]
HER2	41%	26%			No amplification [a]	[14]
	6%	6%	27% [c]			[17]
			70% [d]			[18]

[a] fluorescent in situ hybridization (FISH), 66 cases of which 32 ARMS and 34 ERMS. [b] fusion-negative ARMS. [c] PRMS (pleomorphic RMS). [d] head and neck RMS (29 cases, of which 18 were ERMS, 10 were ARMS and 1 was

Through the study of publicly available microarray experiments, the HER family expression in RMS was compared to that of normal muscle [19]. EGFR expression by ERMS was higher than that of normal muscle. RMS overexpressed HER3 versus normal muscle, with a higher HER3 expression in ARMS than in ERMS. HER4 was downmodulated in RMS versus normal muscle.

3. The Role of HER Family in the Onset and Malignancy of RMS

HER2 amplification was sporadically reported in small series of ERMS [20][21]. In a large RMS series an expressed mutation of HER2 was found in two cases of fusion-negative RMS (mutations R678Q or S310F), corresponding to 1.4% of total RMS cases (3.2% of fusion-negative RMS cases), while no event was found in fusion-positive RMS [7]. Among RMS cell lines listed in the somatic mutation COSMIC database, only TE-441-T (classified as ERMS, even though no characterization is available, see [22]) carries a mutation in the HER2 gene (R432W) [23]. Therefore, for a small fraction of fusion-negative RMS cases the somatic mutation in HER2 gene can be an oncogenic driver.

HER3 can mediate RMS differentiation induced by glial-derived growth factor 2 (a specific ligand of HER3 that stimulates normal myogenesis), in the absence of effects on cell growth [24].

HER3 could also play a protumoral role. The HER3 zebrafish homologue (HES3) is a target gene of the PAX3–FOXO1 chimeric transcription factor resulting from translocation and HES3/HER3 overexpression in fusion-positive RMS was associated with a significantly reduced survival [25]. In the ERMS RD cell line, the induced overexpression of HER3 caused increased cell growth while HER3 silencing determined a decreased growth [19]. The study of an RMS-related miRNA signature, common to all RMS subtypes [26], showed that a commonly downregulated miRNA in RMS is the oncosuppressor miR-22. HER3 transcript contains in its 3′UTR a functional response element to miR-22. The upregulation of HER3 is a mechanism of primary resistance to MEK inhibitors shown by RMS [26].

A transgenic rat HER2/neu allele, activated by point mutation V664E, if coupled to an inactivated p53 tumor suppressor allele, caused high-penetrance genitourinary RMS in male mice [27]. Tumors showed typical markers of RMS (desmin, myosin and a high expression of IGF2) and histologically resembled ERMS. This HER2-driven RMS murine model has an advantage over other RMS murine models due to the predictable site of onset. This allowed us to perform immunoprevention studies: both active and passive immune approaches succeeded in specific delay of RMS onset [28][29]. HER2-driven RMS are triggered at the genitourinary site of male mice by the coincidental increased expression of HER2 and the under-expression of p53. The two genetic alterations foster p53 loss, IGF2 autocriny and overexpression of p19Arf and p21Cip1 [30].

4. HER Family Members as Therapeutic Targets

EGFR is mainly expressed by ERMS cell lines, as well as by primary tumors. In some ERMS cell lines (but not all), EGFR can contribute to tumor growth. Strategies to suppress EGFR activity through the use of neutralizing

antibodies [13][31][32] and transduction of the antisense construct [33] generally caused an impairment of the proliferative ability of EGFR-positive RMS cells, with low/null effect on myogenic differentiation.

HER2 expression level detected in RMS cells by cytofluorometric analysis was sizeable, but it was at least two orders of magnitude lower than that reported for HER2-overexpressing SK-OV-3 human carcinoma cells [34]. The few cases of breast cancer with HER2 mutations (about 1.6%) might be candidates for anti-HER2 inhibitors [35].

HER family members can crosstalk with other RTK members, being involved in the onset of resistance to treatments directed against other receptors for growth factors [36].

The expression of HER family members in RMS cells was exploited to vehiculate toxic agents. This approach could in principle be active even when target antigens are not essential for tumor growth. Immunotoxins targeting HER2 or HER3 did not cause any effect on growth or differentiation of RMS cells [34]. The authors attributed the different efficacy of EGFR versus HER-2/HER-3 immunotoxins to the different endocytic routing of HER receptors, rather than to expression levels of the various target antigens [34].

HER family members expression via RMS could be exploited for use in the targeting of immune cells to tumors by chimeric antigen receptors (CAR). A good safety profile was shown by HER2-CAR-T exploiting the scFv derived from the FRP5 anti-HER2 monoclonal antibody. FRP5-derived HER2-CAR-T cells determined remission in a child with score 3 HER2-positive fusion-negative ARMS, metastatic to bone marrow and refractory to conventional therapy [37]. After relapse, a further remission was determined by HER2-CAR-T administration combined to the checkpoint inhibitor PD-1 blocking antibody. The setup of CAR-directed therapy seems particularly promising, but the immunosuppressive tumor microenvironment likely requires that a combined treatment with checkpoint inhibitors be applied.

Alternative effectors to be transduced with HER2-CAR constructs could be cytokine-induced killer (CIK) cells that are in vitro-expanded immune effectors exhibiting T and NK phenotypes (so-called T-NK) and a non-MHC-restricted cytotoxic ability [38] Engineering of HER2-CAR into cells with NK phenotype of various origin has been proposed as an "off-the-shelf" approach for HER2-expressing RMS [39].

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