Amplification of ERBB2 (HER2) in embryonal rhabdomyosarcoma: A potential treatment target in rare cases?

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Funding information
Governmental Funding of Clinical Research within the National Health Service of Sweden; Swedish Cancer Society; The Swedish Childhood Cancer Fund

Abstract
The ERBB2 gene encodes a receptor tyrosine kinase also known as HER2. The gene is amplified and overexpressed in one-fifth of breast carcinomas; patients with such tumors benefit from targeted treatment with trastuzumab or other drugs blocking the receptor. In addition, ERBB2 has been shown to be amplified and/or overexpressed in a variety of other malignancies. Notably, both alveolar and embryonal rhabdomyosarcoma (RMS), especially in children, often show increased expression of ERBB2. Although high-level amplification of the gene has not been described in RMS, its frequent expression at the cell surface of RMS cells has been exploited for chimeric antigen receptor T-cell (CAR T)-based treatment strategies. We here describe two cases of pediatric, fusion-negative embryonal RMS with high-level amplification of the ERBB2 gene. One patient is currently treated with conventional chemotherapy for a recently detected standard risk RMS, whereas the other patient died from metastatic disease. Both tumors displayed focal amplicons (210 and 274 Kb, respectively) in chromosome band 17q12, with proximal and distal borders corresponding to those typically seen in breast cancer. In both tumors, the ERBB2 amplicon correlated with high expression at the RNA and protein levels. Thus, breast cancer-like ERBB2 amplification is a very rare, but recurrent feature of pediatric RMS, and should be exploited as an alternative treatment target.

KEYWORDS
amplification, ERBB2, rhabdomyosarcoma

INTRODUCTION
Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children. Tumors are morphologically divided into four subgroups, the two largest being alveolar RMS (ARMS) and embryonal RMS (ERMS).1 Whereas ARMS often display a fusion of PAX3 or PAX7 with FOXO1, ERMS are typically fusion-negative, instead being genetically characterized by various combinations of copy number changes (in particular gain of chromosomes 2, 7, 8, 11–13, and 20) and mutations affecting the Hedgehog, RAS, PIK3, or DNA repair pathways.2

Current treatment strategies for RMS include a multimodal approach with a combination of surgery, radiotherapy, and chemotherapy.3 Still, a substantial fraction of patients succumb to their disease, with poor

Received: 20 July 2021 | Revised: 17 August 2021 | Accepted: 18 August 2021
DOI: 10.1002/gcc.22996

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outcome being linked to a number of adverse features such as age > 10 years, fusion-positivity in tumors with ARMS morphology, tumor size, and metastases at diagnosis. Hence, new treatment strategies are needed. One possible approach is to use genetic data to find novel treatment targets that may be present in subsets of the disease. One such candidate is the receptor tyrosine kinase ERBB2 (also known as HER2 or Neu), which has been shown to be expressed at the protein level in 10%–33% of RMS. The expression of ERBB2 is thus of potential use for CAR T-based treatment. Indeed, complete remission has been achieved with CAR T treatment in a child with metastatic RMS that was refractory to standard treatment. The status of the ERBB2 gene in RMS is hence of potential clinical importance, and we here describe two children with ERMS, both displaying ERBB2 amplicons resembling those previously reported in breast carcinomas and other epithelial malignancies.

2 | MATERIALS AND METHODS

2.1 | Patients

2.1.1 | Case 1

A girl aged 2 years and 10 months presented with an 8.5-cm tumor of the vagina. No metastases were seen at diagnosis, and the tumor was considered standard risk. No predisposing variant in DICER1 or TP53 was found. She is currently receiving chemotherapy according to the Cooperative Weichteilsarkom-Studie (CWS) RMS standard risk protocol, and the primary tumor has not yet been resected. Histopathological analysis of the preoperative biopsy showed a submucosal ERMS. IHC demonstrated expression of MYOD1 and myogenin, and retained expression of SMARCB1, but not of cytokeratin. ERBB2 was strongly expressed (Figures 1 and S1A–C).

2.1.2 | Case 2

A 6.5-year-old girl presented with a 5-cm soft tissue tumor that infiltrated the skull base, with two suspected lung metastases at diagnosis. There was also suspected growth into sinus cavernosus. The tumor was considered high risk and she received chemotherapy according to the CWS 2002 protocol in combination with irradiation (1.64 Gy/day, total dose 45.9 Gy). The disease progressed, including to the meninges, during treatment, and the patient died 8 months after diagnosis. Histopathological analysis showed an ERMS (Figure S1F). IHC revealed expression of desmin and myogenin, but not of cytokeratin. ERBB2 was strongly expressed (Figure 1).

2.2 | Whole genome sequencing (WGS)

DNA from the tumor and peripheral blood of Case 1 was extracted using standard methods. Following library preparation with the Illumina TruSeq DNA PCR-Free kit, paired-end sequencing (2 × 75 nt) was performed using Illumina reagents and equipment (Illumina, San Diego, CA). Sequencing depths were 30x for the blood and 90x for the tumor. Preprocessing and variant calling for the WGS data was performed using the ngscore-sarek pipeline v2.6.1. Reads were trimmed of adapter sequences with Trim Galore v0.6.4 and further processed with BWA v0.7.17 and GATK v4.1.7. Variant calling for SNVs was performed using Strelka v2.9.10 and GATK Mutect2 and structural variants were identified using Manta v1.6.0. Copy number calling was done using AlleleCount v4.0.2 and ASCAT v2.5.2 (penalty = 50; gamma = 1). Segments identified by ASCAT were further pruned by adding segments smaller than 10 Kb to the nearest larger segment to avoid germline CNVs and artifacts. Structural variants identified using Manta were annotated by both SnpEff v4.3 and VEP v99.2. Only structural variants annotated by both tools were kept and were analyzed together with the CNV data to create circcos visualization using R package svpluscnn.

2.3 | Single-nucleotide polymorphism (SNP) array analysis

The genomic imbalances in the tumor of Case 2, as identified with an Illumina OmniQuad array, have been reported before. Here, the tumor DNA was re-examined using the Affymetrix Cytoscan HD array (Affymetrix, Santa Clara, CA). The analysis was performed as described. Tumor Aberration Prediction Suite (TAPS) and Rawcopy, with an adaptation for genome build hg38, were used for segmentation of copy number shifts, copy number evaluation, and visualization of the data. ASCAT was further employed to assess purity, ploidy, and copy number levels.

2.4 | mRNA sequencing (RNA-seq) and gene expression analysis of ERBB2

RNA was extracted from frozen tissue from Cases 1 and 2, as well as, from an additional 15 RMS (six ARMS, nine ERMS) without ERBB2 amplification at SNP array analysis. RNA extraction, library preparation, and sequencing of paired-end 150 nt reads were performed as described. For gene expression analysis, RNA-seq data were aligned with STAR-alignment (STAR/2.5.0a) and normalized using Cufflink. The gene expression level of ERBB2 in RMS with ERBB2 amplicons (Cases 1 and 2) was compared with 15 RMS without ERBB2 amplification. The gene expression level of ERBB2 was visualized in a Boxplot diagram using the Quacore Omics Explorer version 3.6 (Quacore AB, Lund, Sweden).

2.5 | Fluorescence in situ hybridization (FISH)

Interphase nuclei from both cases were analyzed using a commercial probe specific for the FOXO1 locus (Vysis, Downers Grove, IL). Interphase nuclei from fresh tumor tissue from Case 1 were also analyzed with a commercial probe set specific for the ERBB2 locus and the centromeric region of chromosome 17 (Vysis). For the detection of ERBB2
in cut FFPE sections, the PathVysion HER-2 DNA Probe Kit II was used (Abbott Molecular, Des Plaines, IL; Ref no. 06N46).

2.6 | Ethics

Ethical approval was obtained from the Swedish Ethical Review Authority (EPN 2017/796) and 2020-03827 for national multicenter approval.

3 | RESULTS

3.1 | Case 1

FISH for FOXO1-rearrangement was negative, but showed one extra copy of the gene. RNA-seq did not show any significant fusion transcript. WGS identified a hyper-diploid tumor genome with multiple numerical and structural imbalances, including deletions in 4p, 4q, 5q, and 9q, and gains involving chromosomes/chromosome arms 5p, 7, 8,
11, 13, 17q, 20, and 21 (Table S1; Figure S1). Notably, two high-level amplicons (~200 copies) were found in 17q: one in 17q12 spanning 210 Kb and affected 10 protein-coding genes, including ERBB2, with the proximal breakpoint between CDK12 and NEUROD2 and the telomeric breakpoint in IKZF3 (Figure 1). The other amplicon mapped to 17q24, contained no protein-coding genes, and spanned 268 Kb with the telomeric breakpoint inside sex reversal region 2 (Table S1). The WGS data showed that the two amplicons were juxtaposed (Figure 1). No significant single-nucleotide variant or insertion/deletion was found. FISH on interphase and metaphase nuclei from cultured cells, as well as on interphase nuclei in cut FFPE sections, suggested that the ERBB2 amplicon was organized in extra-chromosomal structures resembling double minute (dmin) chromosomes (Figures 1 and S1). RNA-seq showed much higher expression of ERBB2 compared to in RMS without ERBB2 amplification (Figure 1). Using a HER2 antibody, strong cytoplasmic expression of ERBB2 was found also at the protein level (Figure 1).

3.2 | Case 2

FISH for FOXO1-rearrangement was negative. RNA-seq did not detect any significant fusion transcript. SNP array analysis disclosed a near-triploid tumor genome with multiple numerical as well as structural imbalances (Table S1; Figure S1). A high-level amplicon (~40–100 copies) spanning 274 Kb was seen in band 17q12; it affected 11 protein-coding genes, including ERBB2, with the centromeric breakpoint in CDK12 and the telomeric breakpoint in IKZF3 (Table S1 and Figure S1). As in Case 1, RNA-seq and IHC for HER2 showed strong expression both at the mRNA and protein levels (Figure 1).

4 | DISCUSSION

Trastuzumab treatment of breast carcinomas with ERBB2 amplification is an iconic example of the benefits of targeted treatment in oncology; around 20% of breast carcinomas show amplification and overexpression of the tyrosine kinase receptor encoded by ERBB2. Although the amplification is associated with poor outcome, patients treated with trastuzumab, or more recent drugs targeting external or internal aspects of ERBB2 (collectively known as HER2-targeted therapies), achieve prolonged progression-free as well as overall survival. Hence, analysis for ERBB2 amplification and/or overexpression with FISH, molecular methods and/or IHC is today part of routine clinical diagnostics in the management of breast cancer patients.

The ERBB2 amplicon in breast carcinomas has been studied in detail. Using oligonucleotide arrays with 244 000 probes, they showed that the amplicon size and borders varied within a 4.4 Mb region, delineated centromERICly by the DDX52 locus and telomERICly by the KRT40 locus. Frequent centromERIC borders were the PLXDC1, FBXL20, MED1, CDK12, and STARD3 genes, and IKZF3, PSMD3, THRA, WIPF2, CDC6, and RARA were frequent telomERIC borders; the most common centromERIC and telomERIC borders were CDK12 and IKZF3, respectively. These breakpoints fit very well with those observed in the two cases of RMS described here (Figures 1 and S1).

Apart from its well-established association with breast carcinomas, ERBB2 amplification is found also in smaller subsets of a variety of other malignancies, for example in carcinomas of the bladder, colorectum, lung, and stomach. While HER2-targeted therapies have shown anti-tumor activity also in these other malignancies, the effects have not been as good as for breast cancer patients, and the effects vary with type of drug. One possible explanation might be that amplification levels vary across tumor types and/or that identical levels of amplification result in different levels of expression depending on tumor type.

Ganti et al. studied a series of 66 children with RMS with regard to immunohistochemical expression and copy number status of ERBB2. Protein expression was seen in one-third of the cases; although the difference was not significant, it was more often seen in ARMS than in ERMS. Using FISH probes specific for ERBB2 in combination with a control probe for a locus in the short arm of chromosome 17, none of the cases had a signal ratio ERBB2:control > 2; thus, none of the cases displayed evidence of ERBB2 amplification. Using immunohistochemistry, Armstead et al. studied a series of 105, predominantly adult, patients with RMS, showing a lower overall (9%), as well as subtype-specific (6% in both ARMS and ERMS), expression of ERBB2; no association between expression and overall survival was seen. In addition to copy number data, sequencing studies have revealed activating ERBB2 mutations in around 1% of PAX3/7 gene fusion-negative RMS, and in a mouse model Erb2b activation combined with Tp53 mutation can lead to RMS development.

The relatively frequent expression of ERBB2 in RMS, and other pediatric sarcomas, has prompted CAR T-based studies employing ERBB2 as the tumor-associated antigen. Indeed, one child with a fusion-negative, refractory metastatic ARMS that showed high expression of ERBB2 by IHC achieved complete remission with CAR T-based treatment. However, the copy number status of ERBB2 was not reported.

Here we show that strong expression of ERBB2 in RMS occasionally is due to focal high-level amplification, akin to what has been described for breast cancer. Our own data, as well as data from larger series, indicate that amplification of ERBB2 is a very rare event in RMS. However, our findings provide further support for the potential therapeutic significance of ERBB2 assessment in RMS. It may well be that cases with strong, breast cancer-like ERBB2 amplicons are more dependent on ERBB2 signaling than are RMS with ERBB2 expression and no or only low-level gain of the gene. In a similar vein, such tumors might be particularly sensitive to CAR T-based approaches. It is important to emphasize, however, that we currently have no data to support this claim: Case 1 has recently started chemotherapy for standard-risk RMS and the ERBB2 amplicon in Case 2 was not discovered until after the patient’s death from metastatic, therapy-resistant disease.
Further support for ERBB2 constituting an important driver event may be derived from the fact that both cases presented here were fusion-negative and that the tumor (Case 1) examined with WGS did not have any significant SNV. Both cases showed fairly extensive genomic copy number changes, sharing relative gain of material from chromosomes/chromosome arms 5p, 7, 8, 11, 17q, 20, and 21. As these imbalances combined involve thousands of genes, and as all of them have been reported before in varying frequencies of ERMS, it is impossible to speculate on their potential impact on tumorigenesis or on how they might interact with the ERBB2 amplification.

ACKNOWLEDGMENTS
The authors thank Center for Translational Genomics, Lund University and Clinical Genomics Lund, and SciLifeLab for providing sequencing service.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

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