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Simultaneous targeting of insulin-like growth factor-1 receptor and anaplastic lymphoma kinase in embryonal and alveolar rhabdomyosarcoma: A rational choice ☆

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Abstract ***Background:*** Rhabdomyosarcoma (RMS) is an aggressive soft tissue tumour mainly affecting children and adolescents. Since survival of high-risk patients remains poor, new treatment options are awaited. The aim of this study is to investigate anaplastic lymphoma kinase (ALK) and insulin-like growth factor-1 receptor (IGF-1R) as potential therapeutic targets in RMS.

Patients and methods: One-hundred-and-twelve primary tumours (embryonal RMS (eRMS)86; alveolar RMS (aRMS)26) were collected. Expression of IGF-1R, ALK and downstream pathway proteins was evaluated by immunohistochemistry. The effect of ALK inhibitor NVP-TAE684 (Novartis), IGF-1R antibody R1507 (Roche) and combined treatment was investigated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays in cell lines (aRMS Rh30, Rh41; eRMS Rh18, RD).

Results: IGF-1R and ALK expression was observed in 72% and 92% of aRMS and 61% and 39% of eRMS, respectively. Co-expression was observed in 68% of aRMS and 32% of eRMS. Nuclear IGF-1R expression was an adverse prognostic factor in eRMS (5-year survival

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46.9 ± 18.7% versus 84.4 ± 5.9%, $p = 0.006$). *In vitro*, R1507 showed diminished viability predominantly in Rh41. NVP-TAE684 showed diminished viability in Rh41 and Rh30, and to a lesser extent in Rh18 and RD. Simultaneous treatment revealed synergistic activity against Rh41 and Rh30.

Conclusion: Co-expression of IGF-1R and ALK is detected in eRMS and particularly in aRMS. As combined inhibition reveals synergistic cytotoxic effects, this combination seems promising and needs further investigation.

1. Introduction

Rhabdomyosarcoma (RMS) is an aggressive soft tissue sarcoma. Although it is relatively rare with an estimated incidence of 4.5 per million, it is the most common paediatric soft tissue sarcoma, and accounts for 3–7% of all malignancies in children.[1] Its two most common forms are embryonal (eRMS) and alveolar RMS (aRMS). The Children's Oncology Group (COG) reported dramatic increases in 5-year survival on chemotherapeutic regimens between 1972 and 1997 (55–73%).[2,3] However, the prognosis for the high-risk subset of RMS patients (e.g. alveolar histology, lymph node involvement, distant metastases, recurrent disease and higher age) remains poor, not exceeding a 5-year survival of 50%.[4–7] Therefore, there is an urgent need for new therapeutic strategies.

The specific targeting of receptor tyrosine kinases is an upcoming treatment strategy for many tumour types, including sarcomas.[8] The insulin-like growth factor (IGF) system is one of the most extensively studied drugable target systems in sarcomas over the past decade.[9] As IGF pathway signalling is believed to play an important role in oncogenesis and progression of RMS, this seems a potential treatment target in these rare sarcomas.[9,10] This is supported by overexpression of both IGF-1 receptor (IGF-1R) and mainly IGFII in RMS tumours, cell lines and xenograft models.[11–14] Furthermore, IGF pathway inhibition by antisense and small interfering RNA, monoclonal antibodies (mAbs) and small molecule tyrosine kinase inhibitors (TKIs) against IGF-1R was shown to result in decreased RMS growth *in vitro* and *in vivo*.[15–18]

Despite promising preclinical evidence of an anti-tumour effect of IGF-1R inhibitors in RMS, the results of clinical trials remain unsatisfactory because of the modest and temporarily anti-tumour effect.[19–21] Since altered activation of the same intracellular survival pathways via alternative receptors was observed upon IGF-1R directed treatment,[22–25] simultaneous targeting of these receptors could be a potential strategy.[26] We hypothesise that the anaplastic lymphoma kinase receptor (ALK) is a potential candidate for simultaneous therapy, as high expression rates were observed previously,[27,28] and as ALK downstream activation overlaps that of IGF-1R, involving the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways.[29]

The aim of the current study is to investigate the (co-)expression of IGF-1R and ALK in RMS tissue samples and correlate this with outcome in a representative multicenter cohort study. Furthermore, we investigate the effect upon (simultaneous) targeting of IGF-1R and ALK in RMS *in vitro*.

2. Patients and methods

2.1. Patients and tumour samples

Tumour material consisted of 112 therapy-naïve biopsies (86 eRMS, 26 aRMS) retrieved from the authors' (referral) files (UEF, AJHS) and PALGA, the nationwide network and registry of histo- and cytopathology in the Netherlands. The current cohort largely overlaps the cohort as described in our previous publication.[27] Clinical characteristics are summarised in Table 1. Tissues and follow-up data were retrieved according to the Dutch Code on Proper Use of tissue (http://www.federa.org/gedragscodes-codes-conduct-en). RMS diagnosis was reviewed and reclassified by an expert pathologist (UEF), based on criteria according to the World Health Organisation (WHO) classification.[30]

Tumour specimens were collected on tissue microarrays (TMA), containing 1–3 cores of 1–3 mm diameter for each sample.

2.2. Cell lines

RMS cell lines (RD, Rh18, Rh30 and Rh41) were generously provided by Dr. Peter Houghton of the Pediatric Preclinical Testing Program (Columbus, OH). RD cells were cultured in DMEM medium (PAA Laboratories GmbH, Pasching, Austria), Rh18 cells in McCoy's 5A medium (Lonza Benelux BV, Breda, the Netherlands) and Rh30/Rh41 cells in RPMI 1640 medium (PAA Laboratories GmbH). All media were supplemented with 10% fetal bovine serum (PAA Laboratories GmbH) and 1% Pen-Strep (Lonza Benelux BV) and cells were cultured in a humidified atmosphere of 5% CO_2/95% air at 37 °C. For immunohistochemistry (IHC) analysis, cells were fixed with Unifix (Klinipath, Duiven, The Netherlands) and processed into AgarCytos.