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Research Article

Irradiation-induced angiosarcoma and anti-angiogenic therapy: A therapeutic hope?

Amalia Azzariti[a,*,1], *Letizia Porcelli*[a,1], *Anita Mangia*[b], *Concetta Saponaro*[b], *Anna E. Quatrale*[a], *Ondina S. Popescu*[c], *Sabino Strippoli*[d], *Gianni Simone*[c], *Angelo Paradiso*[e], *Michele Guida*[d]

[a]*Clinical and Preclinical Pharmacology Laboratory, National Cancer Research Centre, Istituto Tumori Giovanni Paolo II, Viale O. Flacco, 65, 70124 Bari, Italy*
[b]*Functional Biomorphology Laboratory, National Cancer Research Centre, Istituto Tumori Giovanni Paolo II, Viale O. Flacco, 65, 70124 Bari, Italy*
[c]*Department of Pathology, National Cancer Research Centre, Istituto Tumori Giovanni Paolo II, Viale O. Flacco, 65, 70124 Bari, Italy*
[d]*Medical Oncology Unit, National Cancer Research Centre, Istituto Tumori Giovanni Paolo II, Viale O. Flacco, 65, 70124 Bari, Italy*
[e]*Experimental Medical Oncology, National Cancer Research Centre, Istituto Tumori Giovanni Paolo II, Viale O. Flacco, 65, 70124 Bari, Italy*

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ABSTRACT

Angiosarcomas are rare soft-tissue sarcomas of endothelial cell origin. They can be sporadic or caused by therapeutic radiation, hence secondary breast angiosarcomas are an important subgroup of patients. Assessing the molecular biology of angiosarcomas and identify specific targets for treatment is challenging. There is currently great interest in the role of angiogenesis and of angiogenic factors associated with tumor pathogenesis and as targets for treatment of angiosarcomas. A primary cell line derived from a skin fragment of a irradiation-induced angiosarcoma patient was obtained and utilized to evaluate cell biomarkers CD31, CD34, HIF-1alpha and VEGFRs expression by immunocytochemistry and immunofluorescence, drugs cytotoxicity by cell counting and VEGF release by ELISA immunoassay. In addition to previous biomarkers, FVIII and VEGF were also evaluated on tumor specimens by immunohistochemistry to further confirm the diagnosis. We targeted the VEGF–VEGFR-2 axis of tumor angiogenesis with two different class of vascular targeted drugs; caprelsa, the VEGFR-2/EGFR/RET inhibitor and bevacizumab the anti-VEGF monoclonal antibody. We found the same biomarkers expression either in tumor specimens and in the cell line derived from tumor. In vitro experiments demonstrated that angiogenesis plays a pivotal role in the progression of this tumor as cells displayed high level of VEGFR-2, HIF-1 alpha strongly accumulated into the nucleus and the pro-angiogenic factor VEGF was released by cells in culture medium. The evaluation of caprelsa and bevacizumab cytotoxicity demonstrated that both drugs were effective in inhibiting tumor proliferation. Due to these results, we started to treat the patient with pazopanib, which was the unique tyrosine kinase inhibitor available in Italy through a compassionate supply program, obtaining a long lasting partial response. Our data

*Corresponding author. Fax: +39 080 5555986.
E-mail address: a.azzariti@oncologico.bari.it (A. Azzariti).
[1] Equally contributed.

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suggest that the study of the primary cell line could help physicians in choosing a therapeutic approach for patient that almost in vitro shows chances of success and that the anti-angiogenetic agents are a reliable therapeutic opportunity for angiosarcomas patients.

Introduction

Angiosarcomas (AS) account for <2% of all soft tissue sarcomas and represent one of the most aggressive forms. They include a heterogeneous set of malignant mesenchymal tumors with a vascular derivation from endothelial cells of blood or lymphatic vessels [1].

Potentially arising in many anatomic sites and organs, they may be sporadic or the result of exposure to environmental conditions such as radiation, long-term lymphedema (Stewart–Treves syndrome), trauma, chronic infection or chemical agents (e.g., vinyl chloride, arsenic medication, steroids). Despite this variability, AS shares an invasiveness behavior consisting of local recurrence and an early hematogenous and lymphogenous dissemination.

Clinical presentations of AS are heterogeneous and include superficial and visceral forms. Prognosis depends on the precocity of the diagnosis and the extension of the disease. For advanced disease prognosis remains poor with a progression-free survival of about 4 months and overall survival of about 8 months [2].

Regarding therapeutic options, large resection, followed if possible by adjuvant radiotherapy, is the cornerstone of curative intent treatment of localized forms [3]. For metastatic or locally advanced AS, current cytotoxic agents have a very modest impact on the disease with doxorubicin and paclitaxel providing the better results in terms of higher response rate and longer progression-free survival [4].

Recently, a strong body of biological evidence regarding the key role of angiogenesis has been accumulated in this particular type of sarcoma, supporting further investigations to explore the role of anti-angiogenic agents as an alternative therapeutic option to chemotherapy.

Nevertheless, preliminary clinical experiences with anti-angiogenic drugs reported modest and conflicting results [5,6]. Given the limited number of cases and the heterogeneity of the study populations, a useful interpretation of the variable efficacy of these agents may be driven by studies on primary cultures. In particular, AS tissues isolated from patients can be utilized to obtain primary cultures which allow to test the efficacy of the drug and to assess the expression of possibly predictive biomarkers. Therefore, reliable and relatively rapid pharmaco-sensitivity screening of an individual patient's AS may allow the identification of the best regimen for personalized therapy of the patient starting from the same patient's tumor biology. One of these drug screening tests is based on the utilization of short-term cell cultures which closely resemble the original cancers, mainly as regards features that are responsible for the pharmaco-sensitivity of the tumor.

To offer insight into this process, we describe here the case of the anti-angiogenic responsiveness to the tyrosine kinase inhibitor (TKI) vandetanib/ZD6474 (Caprelsa) and the monoclonal antibody (mAb) bevacizumab (Avastin®) of a primary cell line derived from a woman with an advanced AS arising on an area irradiated for a previous breast cancer. We demonstrated that the in vitro study of the primary cell line allowed us to verify the activity of these two different therapeutic agents addressing the choice of drug with greater chances of success in AS patients.

Materials and methods

Drugs and chemicals

Vandetanib/ZD6474 (Caprelsa) was provided by AstraZeneca Pharmaceuticals (Macclesfield, UK.). Stock solutions were prepared at 20 mM in DMSO and stored in aliquots at −20 °C. Bevacizumab (Avastin®) was commercially available. Further dilutions were made in medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 50,000 UL-1 penicillin and 80 μM streptomycin.

Immunohistochemistry (IHC)

The surgically excised sample was fixed in neutral 10% buffered formalin, embedded in paraffin, then cut at 4-μm thickness and stained with Haematoxylin and Eosin. In addition, 4-μm sections were incubated with CD34 (mouse monoclonal antibody, clone QBEND-10, diluted 1:50; Novocastra Lab. Ltd., UK), Factor VIII (rabbit polyclonal antibody anti-human von Willabrand factor, diluted 1:300 Dako, Denmark), vascular endothelial growth factor (VEGF) (rabbit polyclonal antibody, clone A-20, diluted 1:150; SantaCruz, USA), hypoxia inducible factor-1α (HIF-1α) (rabbit polyclonal antibody, clone H206, diluted 1:50; SantaCruz, USA) as previously described [7,8] and Ki67/MIB1 (mouse monoclonal antibody, clone MIB1, diluted 1:100; Dako, Denmark), overnight at 4 °C. The sections were then incubated with biotinylated linked secondary antibodies for 60 min at room temperature. Slides were developed with 3-amino-9-ethylcarbazole substrate-chromogen (LSAB2 System-HRP; Dako, Denmark) for VEGF and HIF-1α. For CD34, Factor VIII and Ki67/MIB1, 3′,3′-diaminobenzidine tetrahydrochloride (Dako, Denmark) were utilized. Slides were counterstained with haematoxylin and examined by light microscopy. Each batch of staining included a negative control section treated with phosphate-buffered saline instead of primary antibodies. Tumor immunoreactivity was scored by two investigators who participated in this study.

Short-term cell culture. A skin fragment from a AS patient biopsy was established in culture, after obtaining the informed consent. The tissue (100 mg) was washed twice in PBS (2.7 mM KCl, 1.5 mM KH_2PO_4, 0.14 M NaCl, 8.1 mM $Na_2HPO_4 \cdot 7H_2O$) and minced with surgical blades under aseptic conditions and layered in 60-mm-diameter dishes in high-glucose Dulbecco′s modified Eagle′s medium (DMEM) supplemented with 10% (v/v) fetal