

Biological indicators of prognosis in Ewing's sarcoma: an emerging role for lectin galactoside-binding soluble 3 binding protein (LGALS3BP)

Diana Zambelli¹, Monia Zuntini¹, Filippo Nardi¹, Maria Cristina Manara¹, Massimo Serra¹, Lorena Landuzzi¹, Pier-Luigi Lollini², Stefano Ferrari³, Marco Alberghini⁴, Antonio Llombart-Bosch⁵, Enza Piccolo⁶, Stefano Iacobelli⁶, Piero Picci¹, Katia Scotlandi¹

¹Laboratory of Oncologic Research, Istituti Ortopedici Rizzoli, Bologna, Italy

²Cancer Research Section, Department of Experimental Pathology, University of Bologna, Bologna, Italy

³Section of Chemotherapy, Department of Musculoskeletal Oncology, Istituti Ortopedici Rizzoli, Bologna, Italy

⁴Section of Pathology, Department of Musculoskeletal Oncology, Istituti Ortopedici Rizzoli, Bologna, Italy

⁵Department of Pathology, University of Valencia, Valencia, Spain

⁶Department of Oncology and Neurosciences, "G. D'Annunzio" University, Chieti, Italy

Starting from an experimental model that accounts for the 2 most important adverse processes to successful therapy of Ewing's sarcoma (EWS), chemoresistance and the presence of metastasis at the time of diagnosis, we defined a molecular signature of potential prognostic value. Functional annotation of differentially regulated genes revealed 3 major networks related to cell cycle, cell-to-cell interactions and cellular development. The prognostic impact of 8 genes, representative of these 3 networks, was validated in 56 EWS patients. High mRNA expression levels of HINT1, IFITM2, LGALS3BP, STOML2 and c-MYC were associated with reduced risk to death and lower risk to develop metastasis. At multivariate analysis, LGALS3BP, a matricellular protein with a role in tumor progression and metastasis, was the most important predictor of event-free survival and overall survival. The association between LGALS3BP and prognosis was confirmed at protein level, when expression of the molecule was determined in tumor tissues but not in serum, indicating a role for the protein at local tumor microenvironment. Engineered enhancement of LGALS3BP expression in EWS cells resulted in inhibition of anchorage independent cell growth and reduction of cell migration and metastasis. Silencing of LGALS3BP expression reverted cell behavior with respect to *in vitro* parameters, thus providing further functional validation of genetic data obtained in clinical samples. Thus, we propose LGALS3BP as a novel reliable indicator of prognosis, and we offer genetic signatures to the scientific communities for cross-validation and meta-analysis, which are indispensable tools for a rare tumor such as EWS.

Ewing's sarcoma (EWS) is the second most frequent primary tumor of bone, generally occurring in children and adolescents. Genetically, EWS is characterized by the presence of specific chromosomal translocations that fuse the *EWSR1* gene (encoding the EWS protein) on chromosome 22 with various *ETS* genes.¹ The most common fusion, EWS/FLI, is

present in 85% of cases, with other fusions accounting for the remaining cases.²

EWS is very aggressive. It requires either surgery and/or radiation therapy for control of the primary tumor site, along with intensive chemotherapy to treat micrometastatic deposits. These multimodal therapies dramatically improved survival of

Key words: Ewing's sarcoma, lectin galactoside-binding soluble 3 binding protein, prognostic markers, microarray analysis, metastasis

Abbreviations: CDDP: cisplatin; C-NED: continuously not evident disease; DX: doxorubicin; EFS: event-free survival; EWS: Ewing's sarcoma; FBS: fetal bovine serum; IFO: ifosfamide; IMDM: Iscove-Modified Dulbecco Medium; OVS: overall survival; REL: relapsed; LGALS3BP or 90K or MAC2-BP: lectin galactoside-binding soluble 3 binding protein; SAM: significance analysis of microarray; TMA: tissue microarrays; VCR: vincristine

Additional Supporting Information may be found in the online version of this article.

Grant sponsor: European Commission; **Grant number:** PROTHETS grant number 503036; **Grant sponsor:** EUROBONET;

Grant number: 018814; **Grant sponsors:** Italian Association for Cancer Research, the Ministry of Health (Programma Integrato Oncologia 2006); **Grant number:** RFPS-2006-3-340280

DOI: 10.1002/ijc.24670

History: Received 6 Nov 2008; Accepted 19 May 2009; Online 19 Jun 2009

Correspondence to: Katia Scotlandi, M.O. "Growth Factors and Receptors," Laboratory of Oncologic Research, Istituti Ortopedici Rizzoli, Via di Barbiano 1/10, 40136, Bologna Italy, Fax: +39-051-6366761, E-mail: katia.scotlandi@ior.it