IMMUNOHISTOCHEMICAL EXPRESSION OF CD133 STEM CELL MARKER IN PRIMARY OSTEOSARCOMA

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List of Abbreviations

ABC	ATP-binding cassette trasporter
AC133	Antibody clone 133
AIP	Aptaf-1 interacting protein
AJCC	American Joint Committee on Cancer Staging
Aptaf-1	Apoptotic protease activating factor 1
ATP	Adenosine triphosphate
Bcl2	B-cell lymphoma
Bcl-XL	B-cell lymphoma-extra large
BCRP	Breast cancer resistance protein
CD133	Cluster of Differentiation molecule 133
CIS	Cisplatin chemotherapeutic agent
CSCs	Cancer stem cells
CT	Computed Tomography
CXCR4	C-X-C chemokine receptor type 4 stem cell marker
DAB	Diaminobenzidine
DNA	Deoxyribonucleic acid
DOX	Doxorubicin chemotherapeutic agent
EpCAM	Epithelial cell adhesion molecule
ES	Embryonic stem cell
ESA	Epithelial specific antigen molecule
FC	Flow cytometry
FLIP	FLICE-inhibitory proteins
GBM	Glioblastoma multiforme

H&E	Hematoxylin and Eosin stain
HS	Highly significant
IAP	Inhibitors of apoptosis proteins
IF	Immunofluorescence
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal stem cell
MTX	Methotrexate chemotherapeutic agent
NS	Non significant
OS	Osteosarcma
PBS	Phosphate - buffered saline
PET	Positron Emission Tomography
PFS	Progression-free survival
Pgp	P-glycoprotein
RB1	Retinoblastoma 1 gene
RNA	Ribonucleic acid
ROC	Receiver operating characteristic curve
RT-PCR	Real-time polymerase chain reaction
S	Significant
Saos2	Sarcoma osteogenic cell line 2
SD	Standard deviation
SEER	Surveillance, Epidemiology and End Results
SP	Side population cell
TNI	Tumor Necrosis Index
TNM	Tumor size, Lymph node and Metastasis Staging
WHO	World Health Organization

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	cytoplasmic CD133 stain in about 85% (CD133 X100).	

Introduction

Osteosarcoma (OS) is the most common primary, non-hematologic bone malignancy in childhood and adolescence, comprise almost 60% of the common histologic subtypes of bone sarcomas **(Tirino et al., 2011).**

Sarcomas are widely believed to develop as a result of genetic mutations in mesenchymal progenitor/stem cells. But the accurate cellular origin of most of these tumors remains unknown **(Tang et al., 2008).**

The cancer stem cells (CSCs) hypothesis predicts that only a small subset of cells within a tumor is capable of initiating a new tumor and sustaining its growth **(Rosen et al., 2009)**. These CSCs are thought to divide asymmetrically, producing an identical daughter stem-like cell and a more differentiated cell, which upon subsequent divisions generates the vast majority of the tumor bulk **(Gibbs et al., 2011).**

The identification of human CSCs brought a hope for tumor management. Searching for specific surface markers on CSCs is the key to further investigate tumorigenesis, metastasis, recurrence and prognosis of tumors (**Zhang & Li, 2010)**.

Specific cell surface markers required to isolate CSCs in solid tumors have not yet been well established (**Neves & Freitas, 2010)**.

Many studies suggested that CD133 was a specific surface marker for stem cells and CSCs (**Zhang & Li, 2010)**. Its interest as a cancer stem marker has grown dramatically since it appeared that it was able to identify a cancer initiating subpopulation in colon **(Gibbs et al., 2005)** and in hepatocellular carcinoma (**Ponti et al., 2005).** Moreover, CD133 cells have also been found melanoma (**Ricci-Vitian et al., 2007)** and in brain (**Tirino et al., 2008).**

CSCs have been already identified in bone sarcomas (**Neves & Freitas, 2010)**. Cell cultures, from biopsy samples of primary osteosarcoma and chondrosarcoma tumors, were analyzed and revealed the presence of a subset of cells that displayed a positive reaction for mesenchymal stem cell markers Stro-1, CD105 and CD44 (**Gibbs et al., 2005)**. In another study, CSCs were detected and characterized based on a CD133-positive profile in established osteosarcoma cell lines **(Tirino et al., 2008)**. Nevertheless, none of the markers used are exclusively expressed by CSCs (**Neves & Freitas, 2010)**.

Surgery, when applied early, can be curative, and preoperative chemotherapy has been shown to be effective in the management of localized chemosensitive tumors, significantly increasing the proportion of long-term survivors **(Tirino et al., 2011).** However, the CSCs are naturally resistant to most current chemotherapy due to their quiescent nature. This may explain why traditional chemotherapies can initially reduce the majority of the tumor bulk but fail to eradicate it in