

# Expression of VEGF, CXCR4 and CD133 in Osteosarcoma

Elka S. Garcia

Faculty: Shang-You Yang

*Department of Biological Sciences, Fairmount College of Liberal Arts and Sciences*

**Abstract.** Osteosarcoma is the most common primary malignant bone tumor seen in orthopaedic practice. Despite intensive treatments of chemotherapy and surgical excision, the current five-year survival rate is only 60% to 78%. We are investigating the correlation of vascular endothelial growth factor (VEGF) expression with osteosarcoma growth and metastasis and trying to explore a therapeutic strategy by blocking VEGF. Recent studies have suggested that a subpopulation of cancer stem cells (CSCs) play a role in cancer survival and metastasis. The purpose of my project is to examine if regulating the function of VEGF can change the population of CSCs in osteosarcoma. Using polymerase chain reaction and immunocytochemistry techniques, we will examine the expression of VEGF, CXCR4 and cancer stem cell marker CD133 at transcriptional and post-translational levels among different VEGF-expressing osteosarcoma cells. We will examine the tumor cell growth patterns and compare the subpopulation ratio of CD133+ cells after VEGF blockage.

## 1. Introduction

Osteosarcoma is the most common malignant bone tumor that affects adolescents and young adults during periods of rapid skeletal growth [1]. The precise mechanism of osteosarcoma remains unclear. However, research has found that tumor reemergence and metastasis are associated with vascularization. A tumor that can initiate the growth of a blood supply is likely to grow rapidly and become more aggressive, metastasize, and ultimately lead to death. Pro-angiogenic factors such as vascular endothelial growth factor (VEGF) are up-regulated in tumors and linked with poor prognosis [2]. Studies in our lab and others suggested that targeting the VEGF signaling pathway in osteosarcoma may therefore improve survival in conjunction with current treatments [3,5]. Recent studies revealed that there are small populations of cancer stem cells (CSCs) present in many types of cancers that can self-renew, proliferate, differentiate, and sustain malignancy [6]. And these cells are usually resistant to conventional chemotherapy. CD133 is identified as one of the specific normal or CSCs markers and is often used to identify the subpopulation of the CSCs in solid cancers including osteosarcoma [6,8]. The objective of this study is to examine the therapeutic influence of VEGF blockage to the CD133+ osteosarcoma CSCs. Our hypothesis is that there will be less therapeutic response in CD133+ cells to VEGF blockage treatment.

## 2. Experimental Methods

Three osteosarcoma cell lines with distinct VEGF expressions (CRL-1423, 1543, and 1547) were cultured in 6-well culture plates (for PCR) or 8-well chamber slides (for ICC) at 37°C in 5%CO<sub>2</sub> for 24 hours before divided into 2 groups: normal control group and the treatment group with a VEGFR inhibitor (SU4312) to diminish effects of VEGF. Cells were harvested at 1, 4, 24, and 72 hours after treatment.

Total RNA extraction was performed following the procedures of a Tel-Test commercial kit. The quantity and purity of the RNA was determined by a spectrophotometer at 260nm and 280nm. Reverse transcription and real time polymerase chain reaction (PCR) for the expression of the target genes (VEGF, CXCR4, and CD133, with

18S as internal control) was carried out using StepOnePlus® Real-Time PCR System according to manufacturer's instructions and published technique [9]. Immunocytochemical (ICC) staining was performed to illustrate CD133+ cells at end of the VEGF blockage experiment. Photomicrographs were taken and a computerized image analysis system (ImagePro+) was used to quantify the positive stains.

## 3. Results

Real-time PCR was used to reveal the gene expressions of VEGF, CXCR4, and CD133 in the three osteosarcoma cell lines. These cells possessed distinct VEGF expression levels, with strongest VEGF expression in 1423 cells, and weakest in 1543 cells. The data clearly demonstrated that CD133 expression levels were negatively correlated with the VEGF expression, 1543 cells exhibited the significantly higher level of CD133 than the rest of two cells. In addition, the data also suggest that VEGF expression positively correlated with CXCR4 expression.

Following VEGFR inhibition with SU4312, the cell proliferation pattern was dramatically decreased over time. Immunocytochemical staining against CD133 confirmed the presence of CD133+ cells in osteosarcoma cells. There were higher CD133 positive signal ratios in 1423 cells at all time points after SU4312 treatment. Additionally in 1423 cells, CD133 expression increased from 1 hour through 72 hours when VEGF was blocked. In originally low VEGF-expressing 1543 cells, further blockage of VEGFR did not change the proportion of CD133+ cells at 24 hour and 72 hour time points, although the total cell numbers were decreased at 24 hours.

#### 4. Discussion

Through microCT and X-ray, pathological assessment, and in vitro examinations, we were able to confirm a decrease in tumor growth and tardiness in lung metastasis when treated with VEGF inhibitor [3]. Another factor that is currently of interest due to its role in the recruitment of leukocytes to sites of inflammation is CXCR4. Research has confirmed that CXCL12, the only recognized ligand for CXCR4, is expressed at high levels in the lung, which is the common metastatic site for osteosarcoma. Therefore, we proposed that by targeting CXCR4/CXCL12 complex, tumor invasion and metastasis will be inhibited [10]. As of now, the linkage between CD133 expression and prognosis of osteosarcoma is yet unknown. In this study, we analyzed the correlation of CD133+ cells with VEGF expression. We proposed that a small subset of CD133+ cells have different VEGF activity. Preliminary data from this study using PCR technique have revealed that VEGF expression was positively correlated with CXCR4 expression, but negatively correlated with CD133 expression. It appears that ICC data complements our PCR data showing that blocking VEGF may up-regulated the CD133+ cells. Osteosarcoma CD133+ cells, as CSCs, may potentially respond to the low VEGF signal and differentiate. Further investigations are warranted to examine the molecular mechanism of this phenomenon and determine its clinical significance.

#### 5. References

- [1] C. A. Arndt, P. S. Rose, A. L. Folpe and N. N. Laack, Common musculoskeletal tumors of childhood and adolescence. *Mayo Clin Proc* 87, 475-487 (2012).
- [2] J. Folkman, Role of angiogenesis in tumor growth and metastasis. *Semin Oncol* 29, 15-18 (2002).
- [3] D. Yin, T. Jia, W. Gong, H. Yu, P. H. Wooley, M. P. Mott and S. Y. Yang, VEGF blockade decelerates the growth of a murine experimental osteosarcoma. *Int J Oncol* 33, 253-259 (2008).
- [4] J. Lammler, M. Fan, H. G. Rosenthal, M. Patni, E. Rinehart, et al. Expression of Vascular Endothelial Growth Factor correlates with the advance of clinical osteosarcoma. *Int Orthop* 36, 2307-2313 (2012).
- [5] L. Angelov, B. Sathya, L. Roncari, G. McMahon and A. Guha, Inhibition of angiogenesis by blocking activation of the vascular endothelial growth factor receptor 2 leads to decreased growth of neurogenic sarcomas. *Cancer Res* 59, 5536-5541 (1999).
- [6] V. Tirino, V. Desiderio, R. d'Aquino, F. F. De, G. Pirozzi, A. Graziano, U. Galderisi, C. Cavaliere, R. A. De, G. Papaccio and A. Giordano, Detection and characterization of CD133+ cancer stem cells in human solid tumours. *PLoS One* 3, e3469 (2008).
- [7] X. Jiang, Y. Gwyne, D. Russell, C. Cao, D. Douglas, L. Hung, H. Kovar, T. J. Triche and E. R. Lawlor, CD133 expression in chemo-resistant Ewing sarcoma cells. *BMC Cancer* 10, 116 (2010).
- [8] J. Li, X. Y. Zhong, Z. Y. Li, J. F. Cai, L. Zou, J. M. Li, T. Yang and W. Liu, CD133 expression in osteosarcoma and derivation of CD133(+) cells. *Mol Med Rep* 7, 577-584 (2013).
- [9] S. Yang, B. Wu, L. Mayton, C. H. Evans, P. D. Robbins and P. H. Wooley, IL-1Ra and vIL-10 gene transfer using retroviral vectors ameliorates particle-associated inflammation in the murine air pouch model. *Inflamm Res* 51, 342-350 (2002).
- [10] D. Yin, L. Bai, W. J. Hendry, P. H. Wooley and S. Y. Yang, Correlation of decelerated tumor growth and diminished c-myc expression following VEGF blockade to a murine osteosarcoma. *Connective Tissue Oncology Society 15th Annual Meeting*. 25, #38968. (2009).