PERIOSTIN PROMOTES METASTASIS BY ENHANCING ANGIOGENESIS AND LYMPHANGIOGENESIS IN ORAL SQUAMOUS CELL CARCINOMA

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Introduction

Oral cancer incidence in Sri Lanka for males and females (age-standardized incidence) are 15.4/100,000 and 4.5/100,000 respectively (Cancer Registry, 2002). In the prognosis of OSCC, the extent of lymph node metastasis is a major determinant. Attempts to identify the genes involved in metastasis are pivotal for the early prediction of behaviour of OSCC. However, the identity and time of onset of the alterations that endow cancer cells with these metastatic functions are largely unknown. In our previous studies we found that Periostin is a highly expressed gene in the invasive clone which was established from a metastatic lymph node (Kudo et al., 2006) and it enhanced invasive activity and metastasis in oral cancer both *in vivo* and *in vitro* (Siriwardena et al., 2006). Angiogenesis and lymphangiogenesis are important in tumor survival and the process of metastasis. VEGF has been demonstrated to play a critical role in the development of tumor vasculature. VEGF secreted from tumor cells, as well as stromal cells, exerts its angiogenic effects on endothelial cells by the activation of Flk-1/KDR (Folkman 2002). It is well known that OSCC frequently metastasizes to regional lymph nodes. In addition, tumors of Periostin over-expressed cells injected mice had regional and distant metastasis with high vascularity (Kudo et al., 2006). Therefore, in the present study we examined the density of blood vessels and lymph vessels in OSCC and correlated them with Periostin expression.

Material and Methods

Assessment of blood vessel was done by in vitro angiogenesis assay using human umbilical vein endothelial (HUVEC) cells and recombinant Periostin. HUVEC cells were treated with different concentrations of recombinant Periostin protein under specific conditions and after 12 days, the cells were fixed and the tubular score was estimated with the Chalkley count method under a bright-field microscope. All 56 OSCC cases were stained immunohistochemically with CD34 endothelial marker and the density was assessed by histomorphometric method. Further, to assess the lymph vessels and VEGF-C expression, OSCC cases were stained with, D2-40 a lymph vessel marker and VEGF-C immunohistochemically and counted the number of lymph vessels in each tumour using Photoshop software. Reverse transcription-polymerase chain reaction (RT– PCR) was performed. Total RNA was isolated from tumor tissues of 31 OSCC and cDNA was

synthesized and amplified in a PC701 thermal cycler. The amplification reaction products were subjected to electrophorese and visualized by ethidium-bromide staining. OSCC cell lines, HSC4 and Ca9-22 were provided by Japanese Collection of Research Bioresources Cell Bank. They were maintained in RPMI-1640 supplemented with 10% heat-inactivated FBS and MSCC-1 and MSCC-Inv1 cell lines were maintained in keratinocyte-SFM under conditions of 5% CO2 in air at 37°C.

Statistical significance was measured for the correlation between Periostin expression and blood vessel density, by the Welch test. The tubular score was also measured by the Welch test. Chi-squared test was used to assess the Periostin expression and VEGF-C.

Results

Periostin promotes capillary formation in a concentration dependant manner. The number and the length of tubules were higher with high concentration of recombinant Periostin protein. Tumors with Periostin expression significantly showed higher number of vessels than those without Periostin expression ($P<0.005$). Periostin over-expressed cells showed higher VEGF-C mRNA levels than control cells. Further, VEGF-C mRNA was expressed in 83% OSCC cases. By immunohistochemical analysis, high expression of VEGF-C was observed in 68.5% OSCC cases. Periostin expressed tumors showed high number of lymph vessels.

Discussion

Periostin promotes angiogenesis, and its expression is significantly correlated with angiogenesis and lymphangiogenesis. These findings suggest that Periostin may play an important role in metastasis of OSCC by promoting invasion, angiogenesis, and lymphangiogenesis. Periostin can be a useful marker to predict metastasis in OSCC and we propose that Periostin may be a therapeutic target in the treatment of OSCC.

References

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