Hypoxia induces translational regulation of VEGFC

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Hypoxia is a major condition for induction of angiogenesis during tumor development, nevertheless its role in lymphangiogenesis remain unclear. In this study, we investigate the role of hypoxia in the translational regulation of lymphangiogenic growth factor VEGF-C.

To determine the presence of IRESes in VEGFC mRNA, we cloned the 5’ untranslated regions of murine and human VEGF-C mRNA in bicistronic lentivectors. First data found the presence of an IRES on VEGF-C mRNA. We found that lymphatic vessels develop in tumors hypoxic gradients in a model of pancreatic human adenocarcinoma xenograft (Capan-1), a syngeneic model of breast cancer (4T1, 67NR) and in lung carcinoma (LLC). We’ve shown, that induction of VEGF-C correlates with hypoxia area development. More importantly, we demonstrated a novel level of regulation of tumor lymphangiogenesis using VEGF-C IRES activation induced by hypoxia both in vitro and in vivo. We found that VEGF-C IRES activity is more upregulated in metastatic cells in draining lymph nodes, known to be highly hypoxic, and demonstrated that this hypoxic microenvironment of tumor cells is efficient to stimulate an alternative regulation of VEGFC expression.

**Lymphatic vessels develop in intratumoral hypoxic gradients**

During tumor development, cells submitted to hypoxia produce both angiogenic and lymphangiogenic growth factors leading to the growth of intratumoral blood and lymphatic vessels. Lymphatic vessels remain the major metastases pathway.

**Hypoxia inhibits cap-dependent**

The majority of cellular stresses such as hypoxia leads to an inhibition of mRNA translation by the classical cap-dependent mechanism. However, several mRNAs (such as VEGFα) are translated by an alternative mechanism mediated by internal ribosome entry sites (IRESes) located in their 5’ untranslated regions.

1) VEGFC is expressed in hypoxic tumor cells

2) There is IRES on VEGFC mRNA

3) VEGFC IRES is activated in vivo

4) Hypoxia induces IRES activation

5) VEGFC IRES is over activated in metastatic tumor cells

6) Hypoxic lymph node environment is sufficient to induce VEGFC IRES activity

To study the effect of microenvironment on lymphatic vessels development, we used different models of carcinoma : Capan-1, a xenograft of human pancreatic adenocarcinoma, 4T1 and 67NR an orthotopic model of mice breast cancer and a subcutaneous model of Lewis lung carcinoma. VEGFC and hypoxic tumor cells have been immunodetected on cryosections. We found an upregulation of VEGFC in hypoxic cells in vivo.

Tumor cells were transduced with a lentivirus expressing a bicistronic mRNA encoding both Renilla luciferase (LucR) and Firefly luciferase (LucF) separated by the VEGF-C and EMCV (viral control) IRES. In vivo VEGFC IRES activity was evaluated.

Luciferase activity was measured in metastatic sentinel lymph nodes. We found an upregulation of VEGFC IRES activity, whereas no activity was found in EMCV control IRES.

Conclusion

This work demonstrates a new level of lymphangiogenic growth factor VEGFC regulation using Internal Ribosome Entry Sites (IRES). We found that hypoxia is a major inducer of VEGFC mRNA translation in tumor cells both in vitro and in vivo. More importantly, we found that VEGFC IRES is even more activated in metastatic cells due to the hypoxic environment of the lymphatic system. Taken together these results provide a new way to target the process of metastases formation.