Hypoxia induces translational regulation of VEGFC

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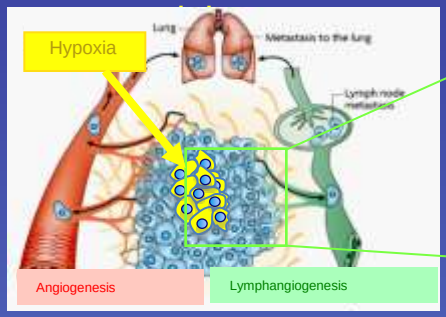
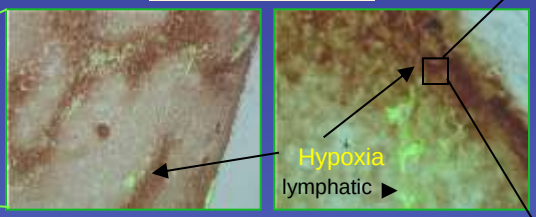
Hypoxia is a major condition for induction of angiogenesis during tumor development, nevertheless its role in lymphangiogenesis remain unclear. In this study, we investigate the role of hypoxia in the **translational regulation** of lymphangiogenic growth factor VEGF-C.
To determine the presence of IRESs 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 syngenic model of breast cancer (4T1, 67NR) and in lung carcinoma (LLC). We've shown, that induction of VEGF-C correlates with hypoxic area developmentt. 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 VEGFC 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

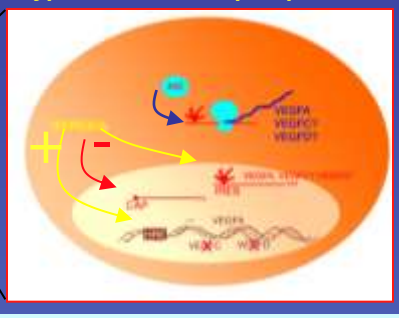
Lymphatic vessels develop in intratumoral hypoxic gradients



Hypoxyprobe/Lyve-1



Hypoxia inhibits cap-dependent

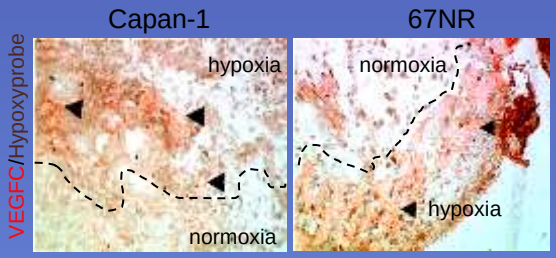


During tumor development, cells submited to hypoxia produce both angiogenic and lymphangiogenic growth factors leading to the growth of intratumoral blood and lymphatic vessels. Lymphatic vessels remain the major metastases pathway.

Intratumoral hypoxia gradients have been visualized using pimonidazole immunodetection. Lyve-1 immunostaining shows lymphatic vessels located in the periphery of hypoxic tumor cells.

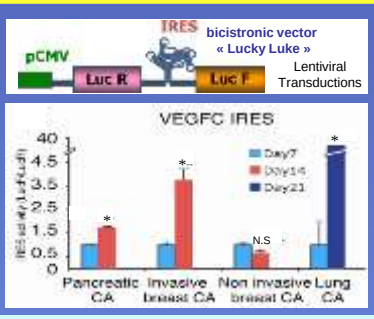
The majority of cellular stresses such as **hypoxia leads to an inhibition of mRNA translation** by the classical cap-dependent mechanism. However, several mRNAs (such as VEGFA) are translated by an **alternative mechanism** mediated by internal ribosome entry sites (**IRESs**) located in their 5' untranslated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

1) VEGFC is expressed in hypoxic tumor cells



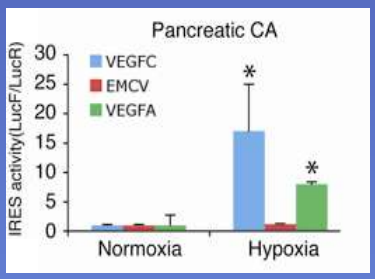
To study the effect of microenvironment on lymphatic vessels development, we used differents models of carcinoma : Capan-1, a xenograft of human pancreatic adenocarcinoma, 4T1 and 67NR an orthotopic model of mice breast cancer and a subcutaneous model of Lewis lung carcinoma. VEGFC and hypoxic tumor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

2) There is IRES on VEGFC mRNA
3) VEGFC IRES is activated *in vivo*



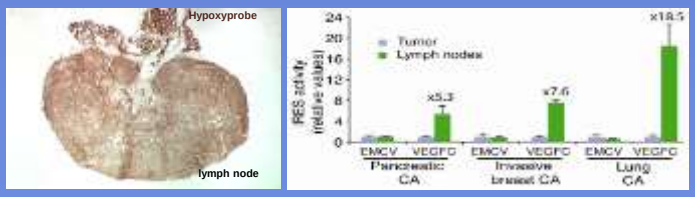
Tumor cells were transduced with a lentivirus expressing a bicistronic mRNA encoding both *Renilla* luciferase (LucR) and *Firefly* luciferase (LucF) separated by the VEGF-C and EMCV (viral control) 5'UTR. In vivo VEGFC IRES activity was evaluated.

4) Hypoxia induces IRES activation



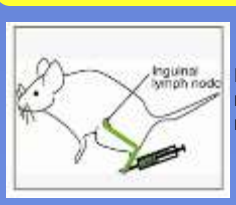
We performed hypoxic stresses on bicistronic vectors transduced cell lines using hypoxia chamber incubator (1% O_2). We demonstrated here that VEGFC and VEGFA IRESes are activated *in vitro* by hypoxia whereas no activation was found for EMCV viral control IRES

5) VEGFC IRES is over activated in metastatic tumor cells

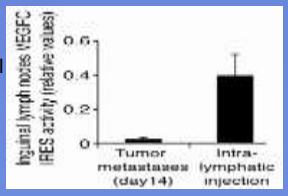


Luciferase activity was measured in metastatic sentinel lymph nodes. We found an upregulation of VEGFC IRES activity, whereas no activity was found in EMCV control IRES.

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



Experimental model of metastases



We injected bicistronic luciferase transduced tumor cells into the lymphatic circulation by injecting cells into foodpads. In that context VEGFC IRES activity was increased twenty fold more after intra-lymphatic injection compared to natural metastases.

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.