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Characterization of the pre-metastatic niche in lymph node, in experimental and clinical settings

A. Noel[1], C. Balsat[1], M. Garcia-Caballero[1], M. Vandevelde[1], S. Blacher[1] and F Kridelka[2]

[1]*Laboratory of Tumor and Development Biology (GIGA-Cancer), University of Liège, Pathology Tower (B23), B-4000 Liège, Belgium.* [2]Department of Pathology, Laboratory of Experimental Pathology, GIGA-Cancer, University of Liege, Liege, Belgium. [3]Department of Obstetrics and Gynecology, Hospital of la Citadelle, Liège, Belgium. [4]*Department of Obstetrics and Gynecology, CHU of Liège, B-4000 Liège, Belgium.*

BACKGROUND AND OBJECTIVES

Clinical issue: **Lymph node status (N+/N-)** is a strong **prognostic factor** of patient's cancer specific survival which is widely used to guide therapeutic decisions. A pelvic and/or para-aortic lymph node dissection remains mandatory in order to confirm the histological nodal status. However, this **surgical staging** is associated to therapeutic **morbidity** (lymphodema). The latter risk is particularly marked when an adjuvant radiation therapy is administrated after the surgery to patients diagnosed N+.

Current view: A specific dialogue between the primary tumor and the lymph node leads to the formation of a supportive microenvironment for metastasis, the **pre-metastatic niche**. A better understanding of pre-metastatic modulations that occur in the **sentinel lymph node** (SLN), the first tumor draining lymph node that remains the initial site of tumor metastasis is needed. A digital image analysis methodology assisted by computer was used to determine objectively whole slide densities and spatial distributions of immunostained structures (D2/40+ lymphatic vessels, CD8, Foxp3, CD20 and PD-1).

Objectives: We aim at getting **new insights** into the **lymphatic vessel dynamics and immune microenvironment** in the sentinel lymph node (SLN) on human early cervical cancers (FIGO stage 1B1, n=50). We also set up an in vivo model reproducing the pre-metastatic lymphangiogenic niche in lymph node.

A. CLINICAL RESULTS

Pre-metastatic lymphangiogenesis

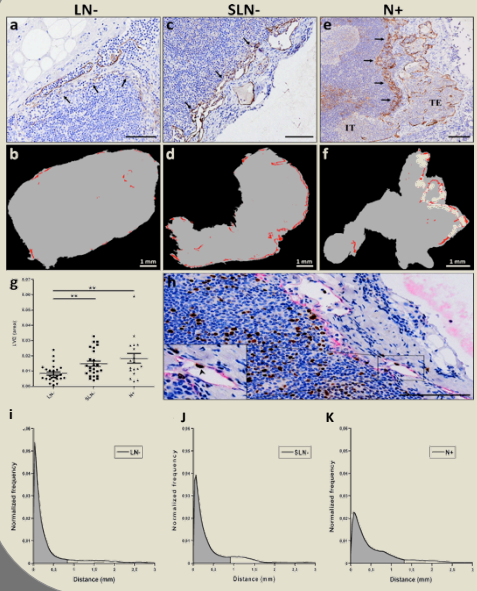


Figure 1: Lymphatic vascular network characterization. (a-f) Illustration of lymphatic endothelial cell immunostaining (D2-40, brown, left column) and corresponding binary image of whole tissue (right column) in LN- (a and b), SLN- (c and d) and N+ (e and f). The subcapsular sinus (arrows), tumor cell emboli within a lymphatic a vessel section (TE in panel e) and invading tumor cells (IT, in panel e) are highlighted. In binary images, lymphatic vessels (red) are detected on whole tissue (grey). Clusters of tumor cells in N+ are represented in white. (g) Lymphatic vessel density measured on whole LN-, SLN- and N+ tissues. (h) Double immunostaining of lymphatic endothelial cells (D2-40, pink) and proliferating cells (Ki67, brown). The insert shows a magnification of a proliferating lymphatic vessel with a ki67 positive endothelial cells (arrow head). The tissue area, in which 90% of the lymphatic distribution are meanly found (grey) is represented. Scale bars on immunostained tissues represent 100µm. **p<0.01.

Pre-metastatic immune modulations

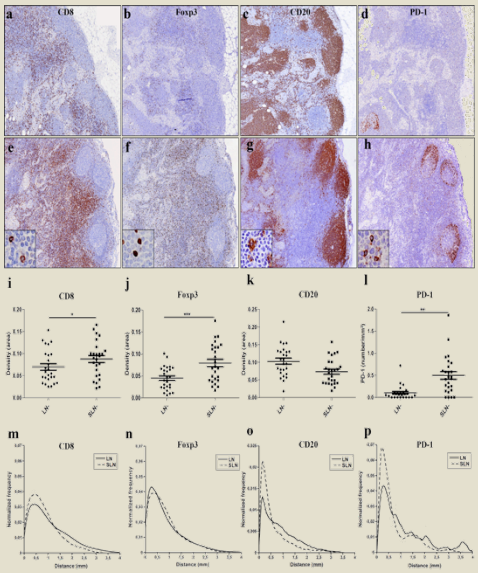
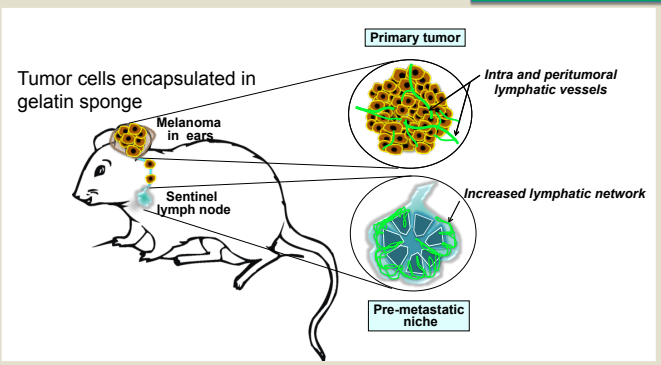
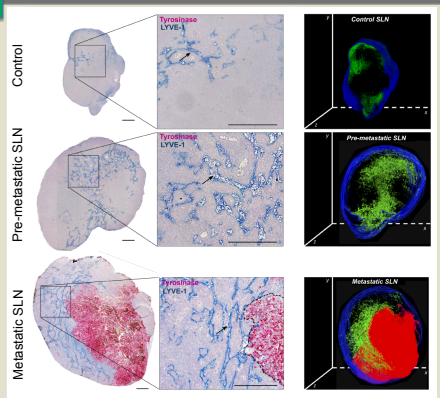


Figure 2: Analysis of the immune response profiles in LN- and SLN-. (a-b) Illustration of the immunostaining of CD8+ T lymphocytes (a and e), Foxp3+ T lymphocytes (b and f), CD20+ B lymphocytes (c and g) and PD-1+ germinal center (d and h) in LN- (first line) and SLN- (second line), (brown). Inserts show the immunostaining of single cells at a higher magnification. (e-l) Computerized quantification of whole slide immunostaining. Densities of immunostaining (e-h) and spatial distribution analysis from tissue edge to tissue center measured in LN- and SLN- (i-l). *P<0.05, **P<0.01, ***P<0.001. Scales bars represent 100 µm.

Balsat *et al*, OncoImmunology, 2017

B. PRE-CLINICAL RESULTS

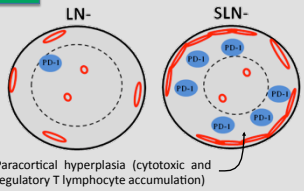




Garcia-Caballero et al, Nature Scientific Report, 2017

CONCLUSIONS

Through a whole slide computer-assisted method, we highlight the presence of a subcapsular lymphangiogenesis that may facilitate the transport of tumor cells from the primary tumor to the SLN-. In addition to the presence of a T regulatory and cytotoxic lymphocyte rich microenvironment in the paracortex of SLN- our data indicate a modulation of humoral immunity (B lymphocytes) in the superficial cortex, which is associated to a subcapsular lymphangiogenesis. All together, our data bring out that, in addition to provide a physical route for tumor cell dissemination, lymphatic vascular network could play a role in the modulation of the humoral pro/anti-tumoral immune response in the SLN prior metastasis.



Summary: A subcapsular lymphangiogenesis (lymphatic vessel = red) occurs prior to tumor cell dissemination. In addition to cytotoxic and regulatory T lymphocyte accumulation in the paracortex, this lymphangiogenesis is associated to humoral modulations: A PD-1+ germinal follicle (blue) accumulation in the superficial area, structure mainly composed by B lymphocytes, is linked to the presence of a subcapsular lymphangiogenesis.