CROI 2015

Cession: P-O2

Poster 723

THE CXCL12/CXCR4-CXCR7 PATHWAY, A TRIO INVOLVED IN KAPOSI'S SARCOMA PATHOGENESIS

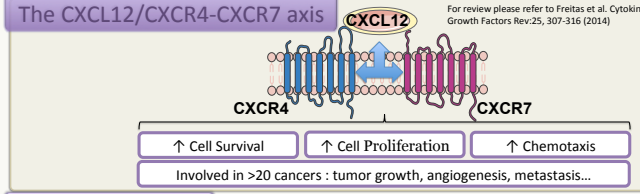
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Introduction

ANRS 154 LENAKAP clinical trial is a single-arm, multicenter, open label, phase II trial, evaluating the efficacy and safety of lenalidomide in Kaposi's sarcoma (KS) associated with HIV infection (AIDS-KS) despite an effective combined antiretroviral therapy (cART) (NCT01282047). KS is a human herpesvirus 8 (HHV-8) -associated disease and remains one of the most common AIDS-associated neoplasia. It is known that cytokines are required for the development of KS, but so far, to our knowledge, no biomarker has been identified. In this sub study, we thus investigate if the CXCL12/CXCR4-CXCR7 pathway, a trio of chemokine and G protein-coupled receptors known to be involved in many cancers, could be a candidate.

The CXCL12/CXCR4-CXCR7 axis



Patients / Methods

Sixteen AIDS-KS cutaneous biopsies (CBs) from 12 men (median age: 47.5y, median KS duration: 6y, median CD4 count: 441/mm^3) were analyzed. Twenty angiomas CBs from 10 patients (9 men, median age: 62.5y) and 21 classic KS CBs from 12 patients (11 men, median age: 75y, median KS duration: 5y, median CD4 count: 696/mm^3) were used as negative and positive controls respectively. The expression of CXCL12, CXCR4, CXCR7 and different markers, i.e. LANA (HHV-8 latency-associated nuclear antigen protein), Ki67 (cell proliferation), TUNEL (apoptosis) and VEGF (angiogenesis), were analyzed on macular, papular and nodular KS CBs and on angiomas CBs by immunohistochemistry (IHC). Protein expressions were quantified using the French Federation of Cancer Centers Sarcoma Group grading systems*. After immunostaining, slides were digitalized using a HPF-NanoZommer® and analyzed using the Visilog® Software. Areas where proteins were highly expressed by endothelial and spindle cells were studied at magnification X 250, so areas of tumor stroma or without an endothelial cellularity were excluded. The count of positive and negative endothelial cells was made at X 400 in 10 successive fields. As some patients had two or more values, we generated 100 bootstrap samples where for each individual, we selected the single value for those with a single evaluation and we randomly selected one value out of the different evaluation for those with two or more evaluations. Then, on each sample, we applied the Mann-Whitney test to compare continuous variables between groups and the Spearman correlation test to examine the associations between continuous variables, and we calculated the mean of p-values and correlation coefficients estimated with each sample.

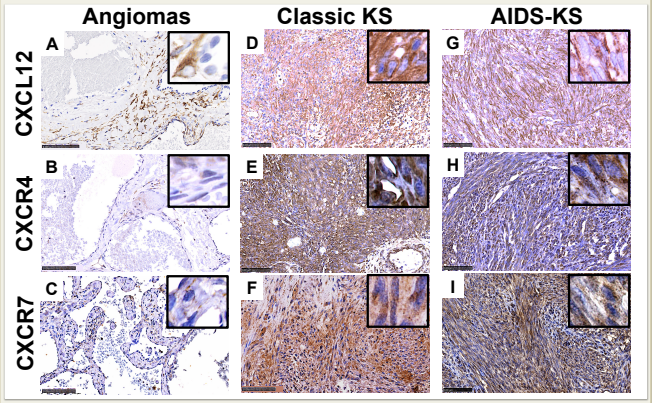
*Reference: Trajoni M, et al. Int. J. Cancer: 33, 37-42 (1984)

Trial and sub study funded and sponsored by ANRS. Trial conducted with the support of Celgene.

Results

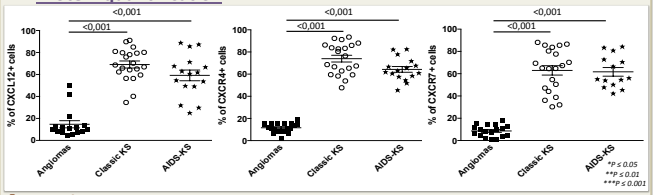
The CXCL12/CXCR4-CXCR7 trio is up regulated in KS lesions

✧Immunostaining



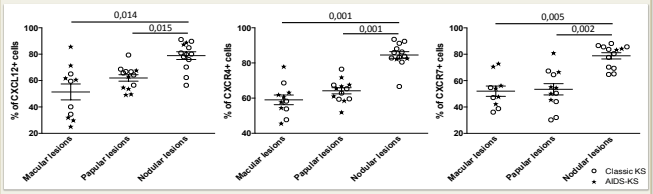
➔CXCL12, CXCR4 and CXCR7 were expressed by some endothelial and lymphocytic cells in angiomas CBs (A-C), and highly expressed by endothelial, lymphocytic and spindle-shaped cells in classic KS (D-F) and AIDS-KS (G-I) CBs, with preferential cytoplasmic localization. (magnification X 20, box: X80)

✧Protein quantification



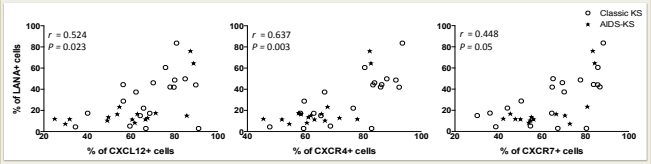
➔Level of CXCL12, CXCR4 and CXCR7 expression are increased in classic-KS and AIDS-KS lesions *versus* angiomas.

CXCL12/CXCR4-CXCR7 is an histological biomarker of KS lesions



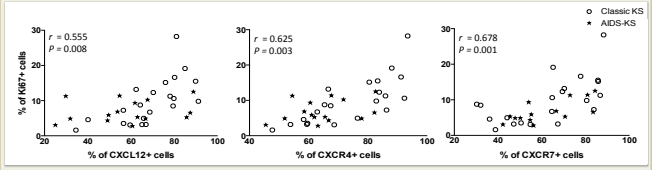
➔CXCL12, CXCR4 and CXCR7 are overrepresented in nodular lesions (tumoral stage) versus papular and macular lesions

CXCL12/CXCR4-CXCR7 expressions are correlated with the presence of latent HHV-8 in KS lesions



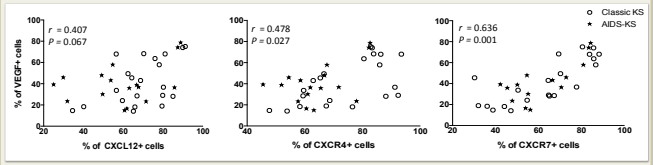
➔There were positive correlations between the trio expressions and LANA detection

CXCL12/CXCR4-CXCR7 expressions are correlated with the proliferation rate in KS lesions



➔There were positive correlation between the trio expressions and Ki67 staining

Expressions of CXCL12 receptors are correlated with the angiogenesis rate in KS lesions



➔There were positive correlations between CXCR4 and CXCR7 expressions and VEGF detection

Conclusions

This is the first *in vivo* study that highlights a concomitant up-regulation of the CXCL12/CXCR4-CXCR7 axis in KS. This deregulation is correlated with physiopathology markers and disease severity. No correlation was found with apoptosis level, patient age, CD4$^+$ T-cells count, lines of chemotherapy and KS disease duration.

➢ **The CXCL12 signaling axis may be involved in this virus-related cancer and could serve as a biomarker of KS severity.**