The inflammasome of tumor cells in colorectal cancer : a potential target to strengthen the Th1/Tc1 response of Tumor Infiltrating T lymphocytes (TILs)

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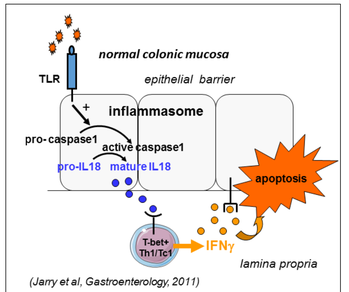
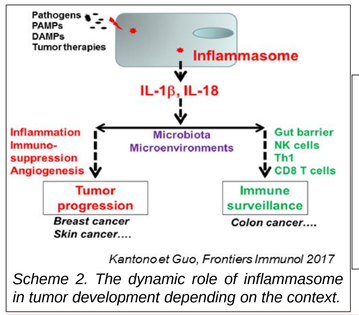
Background

The inflammasome is an innate immunity platform present in normal human intestinal epithelial cells, whose effector protein, caspase-1, can rapidly mature IL-18 and generate a mucosal Th1/Tc1 (IFNγ) response (scheme 1). The role of the inflammasome in cancer remains elusive and controversial. Indeed, it can promote or inhibit tumor progression, depending on the context and organ (scheme 2). In colon carcinogenesis, the inflammasome is mainly studied in mouse models of colitis-associated cancer, a clinical entity distinct from human sporadic colorectal cancer (CRC).

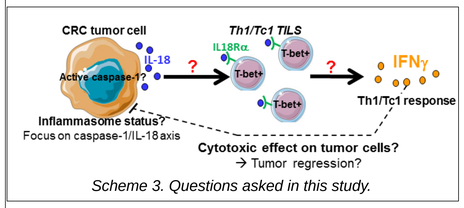
The **aims** of this work were to determine i) the inflammasome status in human CRC tumor cells and ii) its potential modulatory effect on the Th1/Tc1 response of TILs (scheme 3).



Scheme 1. Normal intestinal epithelial cells contain a functional inflammasome.



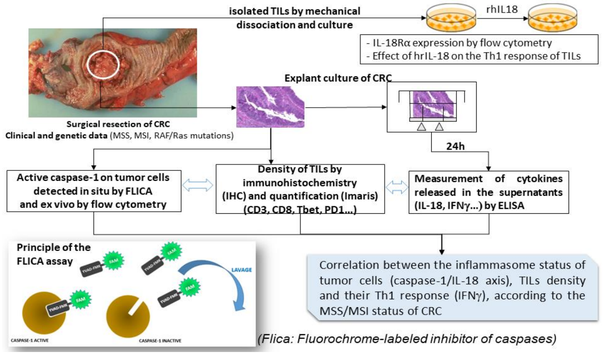
Scheme 2. The dynamic role of inflammasome in tumor development depending on the context.



Scheme 3. Questions asked in this study.

Patients and methods

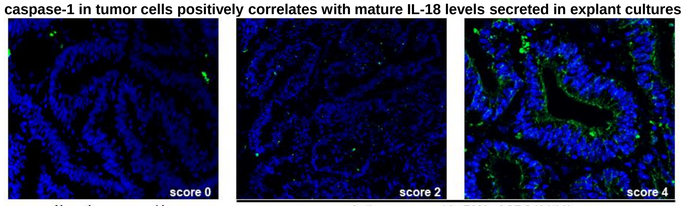
Prospective cohort of 96 CRC patients. Surgical resections of 74 microsatellite stable (MSS) and 22 microsatellite instable (MSI) CRC. (2015-2019, in collaboration with the Pathology, Genetics, Digestive Surgery and Gastroenterology Depts, CHU Nantes, France). **Descriptive and functional studies using *ex vivo* explant cultures of CRC as well as isolated TILs maintained in culture**.

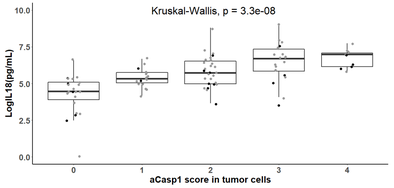


(Flica: Fluorochrome-labeled inhibitor of caspases)

Results

I. Active caspase-1 in tumor cells positively correlates with mature IL-18 levels secreted in explant cultures of CRC





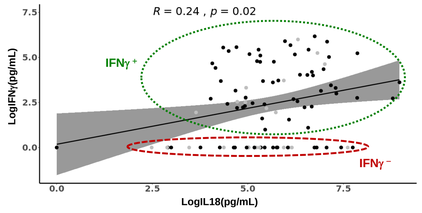
◢ In situ detection of active caspase-1 (aCasp1) in tumor cells by the Flica assay on frozen sections. No active caspase-1 (score 0); Active caspase-1 in 30% of tumor cells (score 2) and 50% (score 4). Green staining shows active caspase-1 (membrane and cytoplasmic staining). Nuclei are stained with Dapi (blue).

◢ Mature IL-18 was assessed in supernatants of 24h CRC explant cultures by ELISA. Each dot represents the mean of triplicate cultures for a given CRC.

Question : can a «functional» inflammasome in tumor cells modulate the Th1/Tc1 response of TILs?

To answer this question, we correlated mature IL-18 and IFNγ levels secreted in the supernatants of CRC explant cultures.

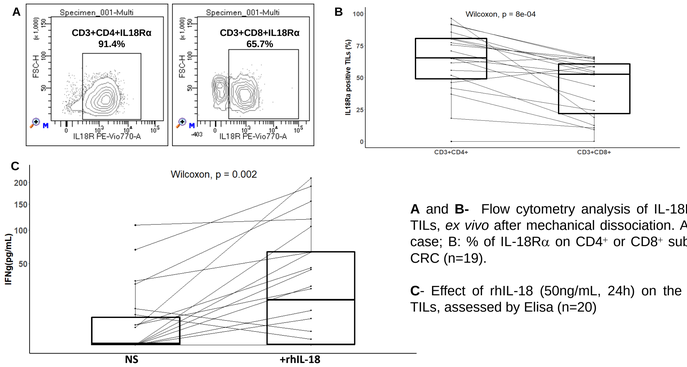
II. Relationship between mature IL-18 and IFNγ released in *ex vivo* explant cultures of CRC



Heterogeneous IFNγ response among CRC patients :
- main group : IL-18+ IFNγ+ (green)
- «paradoxical group» : IL-18+ IFNγ- (red)

Questions :
- Expression profile of IL-18 receptors (IL-18Rα) and effect of recombinant human IL-18 (rhIL-18) on the IFNγ response of isolated TILs from CRC ? (Section III)
- Existence of distinct subgroups of CRC according to the caspase-1/IL-18/IFNγ axis and MSS/MSI status ? (Section IV)

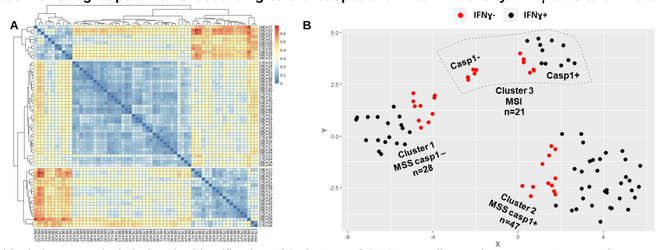
III. TILs isolated from CRC express IL-18Rα and rh IL-18 induces or enhances their IFNγ response



A and **B**- Flow cytometry analysis of IL-18Rα expression on TILs, *ex vivo* after mechanical dissociation. A: a representative case; B: % of IL-18Rα on CD4⁺ or CD8⁺ subsets of TILs from CRC (n=19).

C- Effect of rhIL-18 (50ng/mL, 24h) on the IFNγ response of TILs, assessed by Elisa (n=20)

IV. Identification of subgroups of CRC according to the caspase-1/IL-18/TIL density/ IFNγ axis and microsatellite status

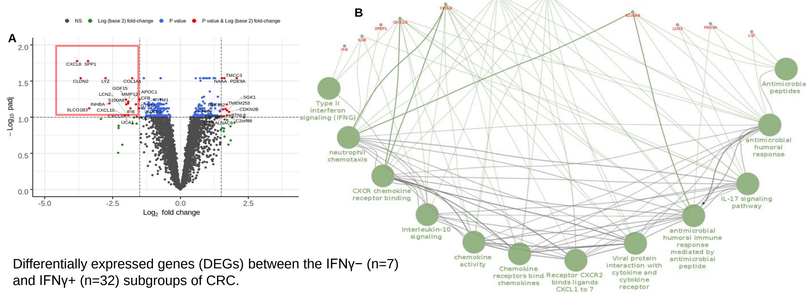


A non hierarchical cluster analysis led to the identification of 3 clusters of CRC according to the caspase-1 status in tumor cells: an expected, cluster of MSI CRC (cluster 3) and 2 clusters of MSS CRC, caspase1⁺ (cluster 2) or caspase1⁻(cluster 1).

A- Heatmap on the dissimilarity matrix based on the Gower metric. **B-** Cluster visualization using a lower dimensional space with *t*-distributed stochastic neighborhood embedding (*t*-SNE). **The "paradoxical" IFNγ⁻ CRC are distributed among these 3 clusters.**

Question: distinct transcriptomic profiles of IFNγ⁻ *versus* IFNγ⁺ CRC ?

IV. Transcriptomic profile of the IFNγ- vs IFNγ+ subgroup of CRC



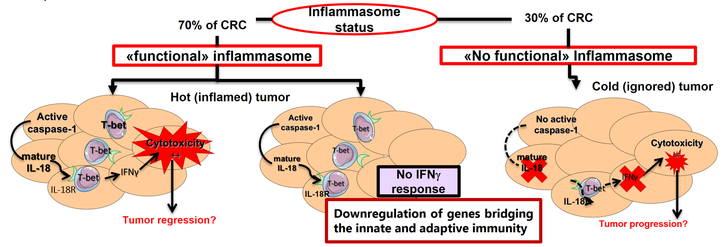
Differentially expressed genes (DEGs) between the IFNγ− (n=7) and IFNγ+ (n=32) subgroups of CRC.

A- Volcano plot of DEGs using the "EnhancedVolcano" package. The red box shows significantly downregulated genes.

B- Pathway enrichment of these downregulated genes in the IFNγ- subgroup highlights a downregulation of genes bridging innate and adaptive immunity.

Conclusions

- This study delineates functional interactions between the inflammasome of tumor cells and TILs adaptive immunity and identifies subgroups of CRC according to the caspase-1/IL-18:TILs desnsity/IFNγ axis.
- Our findings support the inflammasome of tumor cells as a potential target to strengthen the Th1/Tc1 immune response in CRC.



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