Intra-tumoral heterogeneity in colon cancer : cellular and molecular approaches

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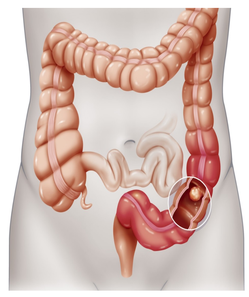
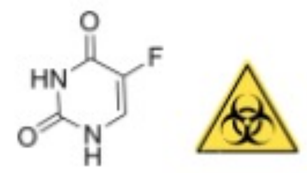
ABSTRACT : Colorectal cancer represents the 2nd cause of cancer mortality in France and worldwide. This pathology is characterized by intra-tumoral heterogeneity highlighted with transcriptomic analyses based on a consensus classification into 4 CMS (Consensus Molecular Subtype) subtypes. This intra-tumoral heterogeneity is found in a panel of colorectal cancer lines. The laboratory uses as a study model the MDST8 mesenchymal type line which is a mixture of cell populations with a CMS1 (70%) and CMS4 (30%) profile. The project will study the phenomenon of tumor heterogeneity in colon cancer via analyses on cellular models, coupled with bioinformatics approaches. We will seek to clarify the transcriptional expression profile which distinguishes MDST8-CMS4 from MDST8-CMS1, to study the signalling pathways and biological processes associated with each subpopulation, to identify one or more biomarkers of each subpopulation in order to to trace them and evaluate the impact of treatment with 5-FU (chemotherapy used as first line treatment for colon cancer) on the different cellular states. This work should make it possible to characterize from a molecular and functional point of view the different cellular states that MDST8 cells can adopt and to assess the impact of cellular stress such as chemotherapy on these different cellular contingents.

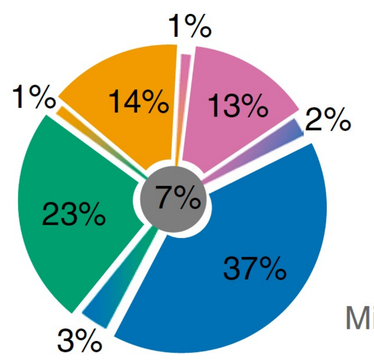


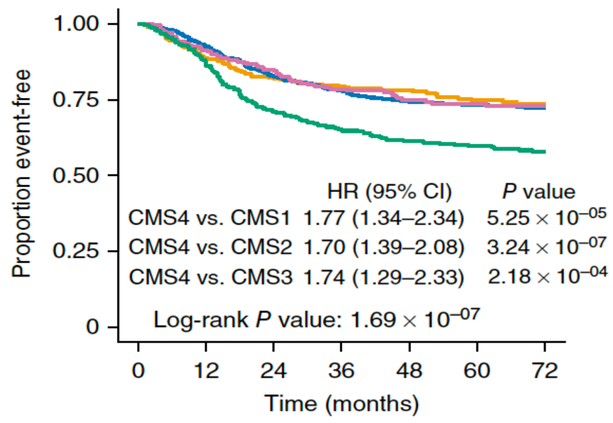


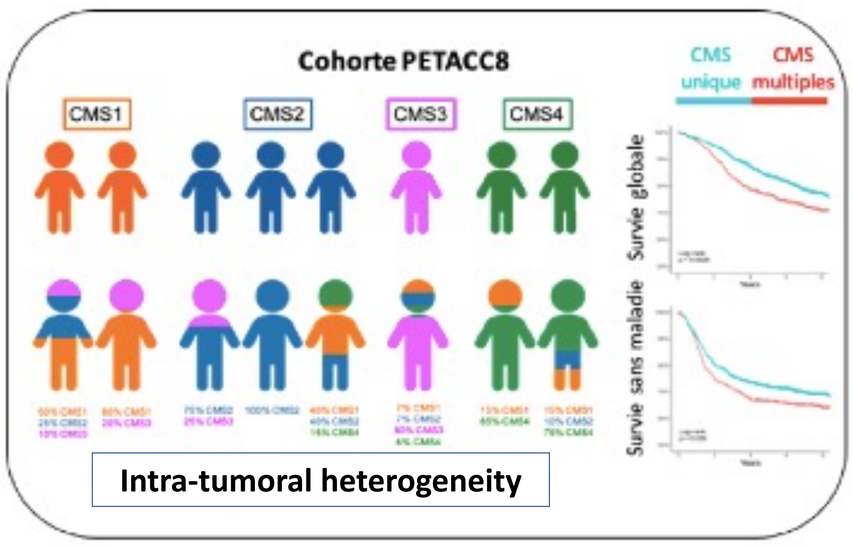
Background of the project :
In colorectal cancer :
-large-scale transcriptomic analyses have revealed molecular diversity underlying a consensus classification into 4 subgroups called CMS1 to 4[1]
-CMS (Consensus Molecular Subtype) subtypes have specific genomic, metabolic or tumor microenvironment characteristics and have prognostic value
-for half of the patients, the tumor can be ascribed to a single CMS, for the other half the tumor is a mixture of 2 or even 3 CMS[2]
-patients for whom several CMS are identified have a worse prognosis than those with a single CMS
-close link between intra-tumoral heterogeneity and chemoresistance phenomena[3]

The PETACC8 cohort revealed tumor heterogeneity in half of the patients. Patients whose tumors correspond to a mixture of CMS have an unfavorable prognosis. This heterogeneity is found in a panel of colorectal cancer lines: each line presents a gene expression profile which corresponds to several CMQs. Our single cell analysis data made it possible to confirm the "bulk" analysis data (same distribution of CMS) and to show for the MDST8 line that the CMSA-CMS4 mixture corresponds to a mixture of cells presenting either a profile CMS1 is a CMS4 profile.

Key questions :
-What characterizes a subpopulation of a given CMS?
-How does a cellular subpopulation defined by its CMS evolve in response to cellular stress such as chemotherapy?

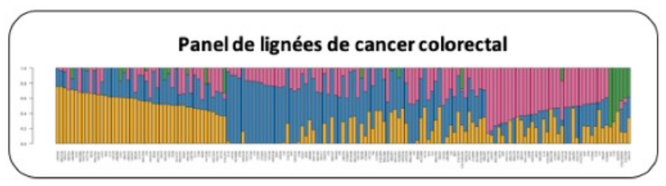


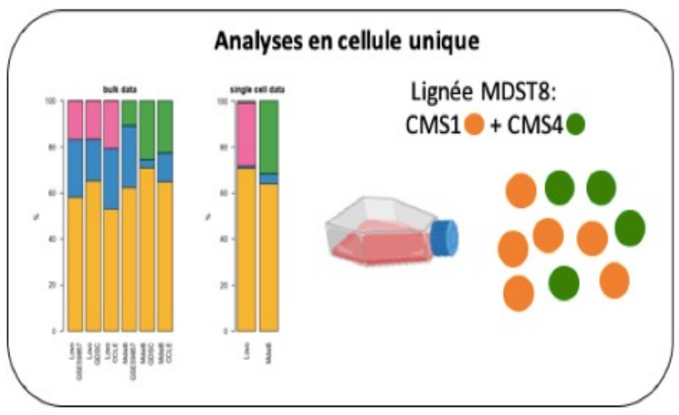


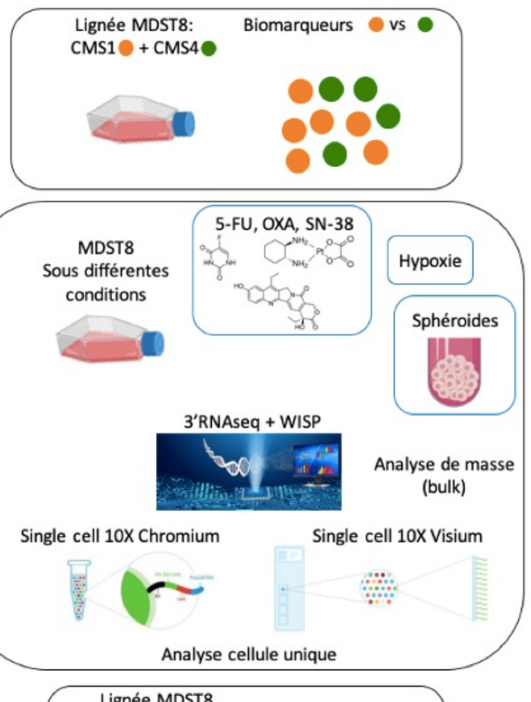


Specific aims :
-specify the transcriptional profile MDST8-CMS1 and 4 by exploiting single-cell analysis data generated in the laboratory
-study the signalling pathways and biological processes in each subpopulation
-identify biomarkers for each subpopulation in order to be able to trace them
-evaluate the impact of 5-FU on different cellular states

The MDST8 lineage (mixture of CMS1-CMS4) will be used as a model system for:
-identify markers of the CMS1 and CMS4 populations
-evaluate the impact of different cellular stresses (chemotherapy, hypoxia) or culture conditions (spheroids) on the CMS1 versus CMS4 distribution, by combining "bulk" analyses and single cell analyses
-develop drug combinations capable of targeting both cell populations

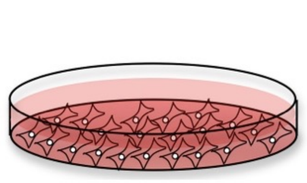


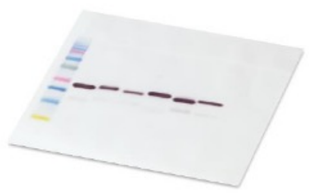




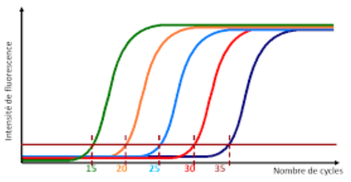
Methodology :
Carrying out cell biology experiments to characterize the different sub- populations of the MDST8 lineage by :
-conventional cell culture
-preparation and analysis of proteins by Western blot
-analysis of protein expression by flow cytometry
-functional analyses by xCELLigence real-time
-transcriptomic profiling analyses (RNAseq) associated with bioinformatics analyses

Analysis of RNAseq data from MDST8 cells having undergone different treatments





Western Blot



RT-qPCR

xCELLigence

References
1. Guinney J et al. (2015) The consensus molecular subtypes of colorectal cancer. Nat Med 21: 1350–1356
2. Marisa L et al. (2021) Intratumor CMS Heterogeneity Impacts Patient Prognosis in Localized Colon Cancer. Clin Cancer Res 27: 4768–4780
3. Marusyk A et al. (2020) Intratumor Heterogeneity: The Rosetta Stone of Therapy Resistance. Cancer Cell 37: 471–484