Talk 9.1

Towards the characterization of single-cell molecular response to ionizing radiation

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The focus of the studies of ionizing radiation damage has historically been centered on the direct impact on DNA due to the important mutagenic effects, especially at higher doses. This outlook tends to minimize the potential for cellular damage on other macro-molecules responsible for cellular functions such as RNA which share a highly similar structure with DNA and can thus be considered at least as vulnerable to ionizing radiation. The analysis of transcriptomes offers insight on the cellular response at a given time when exposed to the stress of ionizing radiation and could thus potentially explain the impact of low doses irradiation on cellular behavior. Third generation sequencing devices have made the study of the RNA metabolism more accessible on a larger scale, allowing for direct sequencing of long-read molecules and the identification of base modifications. More recently, single-cell sequencing has become widely available making the analysis of individual cellular response to external stress possible. The combination of these technologies has opened the way for the study of biological and molecular mechanisms at the scale of the individual cell which have yet to be defined in the interactions with ionizing radiation.

The use of charged particle microbeam @ AIFIRA facility [1] emitting 3 MeV protons offers us the possibility to selectively irradiate subcellular or cellular compartments at controlled doses and observe the impact on an organism's development [2]. We are working with the reference organism Caenorhabditis elegans, a nematode with an identical development among individuals, offering a solid frame of reference for comparative analysis. A protocol of immobilization has been established to reproducibly irradiate selected cells on this organism, allowing us to progress towards single-cell analysis. Our current objective is to complete this protocol with sequencing and bioinformatic tools to produce thorough transcriptomic analysis of ionizing radiation induced damage on individual cells.

The main results obtained using those technologies will be presented :
1 - Irradiations of young worms (L1 stage) on their Z1-Z4 cells which develop to form the gonads and reproductive system. These worms grew with varying levels of vulva's structural anomalies depending on the deposed dose.
2 - Flow cytometry analysis by COPAS on worms exposed to whole-organism irradiation at the L1 stage have produced quantitative results on the dose-dependent induced development delays to reach the adult stage.
3 - Transcriptome analysis by direct sequencing of mRNAs extracted 4 hours after irradiation to identify the cell response to the dose-dependent induced stress in terms of expression, epitranscriptome and base modifications.

References

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