Richiesta 1 Borsa di ricerca - 2025

PROJECT

Bioinformatic analyses of mutational signatures in cancer cells.

OBJECTIVES and BACKGROUND.

A high transcription rate (hyper-transcription) is a hallmark of human cancers. Transcription/replication collisions (TRCs) therefore occur more often in proliferating cancer cells leading to DNA double-strand breakage (DSB), mutations and genome instability. TRCs are a major cause of tumorigenesis and cancer progression. Topoisomerase I (Top1) poisoning can efficiently trigger TRCs, DSBs, mutations and micronuclei, impacting therefore on cancer cell behavior. The fellow will carry out a project with the aim to determine the main mutational patterns induced by Topoisomerase I poisons in cancer and normal cells. The results will reveal novel mechanistic aspects of cancer genome instability that can be exploited to abolish or reduce genome instability resulting in the prevention and/or therapeutic improvements in cancer patients.

RESEARCH ACTIVITIES

The project aims at producing whole-genome sequencing data from a number of cancer cell lines. This approach will allow us to establish any mutational signature linked to head-on and co-directional TRC-dependent. Cancer cells will be treated with CPT for 1 hour to induce TOP1ccs and DSBs. Then, cells will be allowed to recover in drug-free medium for 2-5 cell cycles to accumulate mutations. We will use H1-hESC cells to investigate genomic and epigenomic outcome of TRC resolution during differentiation. High-depth sequencing of multiple single-cell derived subclones will be performedvfor each condition and WGS data from the original isogenic cancer cell line will be used as a reference. In the same cell systems, we will determine gene expression profiles with single-cell RNA-seq technique. We will repeat this approach in a number of cancer cell lines, to explore any cell line-dependent specificity. The results will reveal whether specific mutations or sequence alterations can be associated with TRC-associated DSBs and their specific orientation. Mutational signatures will be analyzed to understand whether they derive from specific repair mechanisms. If we will not detect significant signatures, we will then delete specific repair factor (BRCA2, Rad51, et al.) to impair high-fidelity homology-dependent repair pathways. A similar approach (repair inhibitors or RNAi) will be used to understand the contribution of repair pathway/factors in generating the observed mutational signatures at TRC/DSB regions.

REFERENCES

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