

Al collegio docenti del Dottorato in Medicina Molecolare

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INTRODUCTION

Some of the mechanisms associated with Homologous Directed DNA Repair (HR) are not completely elucidated. It is known that HR is elicited in correspondence of stalled DNA replication forks, such as those induced by DNA-DNA crosslinking agents. Mitomycin C or diepoxybutane (DEB) can cause inter- or intra-strand DNA cross-links and the occurrence of HR can be observed by the dramatic increase of sister-chromatid exchanges in mitotic metaphases. However, it is not well understood whether the HR systems act only on the specific spots of damaged DNA or whether act more globally involving parts of the genome without lesions.

METHODS

In order to investigate these aspects, we studied the mutation rate at the *HPRT* locus of HCT116 cell line treated with vehicle alone or DEB (at the non-toxic dose of 6 μ M) in combination with transfections using control reagents or a mutagenic donor vector with homologies for *HPRT* locus. The number of colonies resistant to 6-thioguanine (6-TG) was measured and compared according to the different combinations of treatments and transfections.

RESULTS

Preliminary results showed that DEB increases the mutation frequency over the background level and that the subsequent administration of the *HPRT*-specific vector can further enhance this frequency. The vector alone does not induce mutations over the background. These data suggest that exogenous DNA homologous to *HPRT* could be specifically incorporated/integrated within an undamaged *HPRT* locus, implying that HR could be chemically induced to act globally at genome level. If this holds true, the HR machinery could be exploited for the modification of genes following its activation by small chemical compounds or drugs.

To allow a faster reading of the results and an easier detection of colonies carrying a specific incorporation at the *HPRT* locus a new mutagenic donor vector is under preparation.

Abstracts e partecipazione a congressi e corsi

Droplet Digital™ PCR Scientific Conference 2019. 21st of May – Centro Congressi Le Benedettine - Università degli Studi di Pisa

Congresso AGI-SIMA Cortona 2019 (26/9 al 28/9 2019) con Abstract e poster:

“Evaluation of homologous recombination rate induced by diepoxibutane.”

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