BASE EXCISION REPAIR MECHANISMS AND RELEVANCE TO CANCER SUSCEPTIBILITY

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Introduction

The base excision repair (BER) pathway is considered the predominant DNA repair system in mammalian cells for eliminating small DNA lesions generated at DNA bases either exogenously by environmental agents or endogenously by normal cellular metabolic processes (e.g. production of oxyradical species, alkylating agents, etc). The main goal of this project is the understanding of the involvement of BER in genome stability and in particular in sporadic cancer development associated with inflammation such as gastric cancer (GC). A major risk factor of GC is the infection by Helicobacter pylori, which causes oxidative stress. Oxidative DNA damage is mainly repaired by BER.

Results

– Genome-wide expression profile of sporadic gastric cancers with microsatellite instability
Gastric cancers with mismatch repair (MMR) inactivation are characterized by microsatellite instability (MSI). In this study the transcriptional profile of 38 gastric cancers with and without MSI was analysed. Unsupervised analysis showed that the immune and apoptotic gene networks efficiently discriminated these two cancer types. Hierarchical clustering analysis revealed numerous gene expression changes associated with the MSI phenotype. Among these the p53-responsive genes maspin and 14-3-3 sigma were significantly more expressed in tumors with than without MSI. A tight immunosurveillance coupled with a functional p53 gene response are consistent with the better prognosis of MSI cancers. The analysis of the expression of genes belonging to DNA repair as a function of the MSI status uncovered other interesting features of GC. MLH1 was confirmed to be a significant event in the development of our MSI gastric tumors that were mostly characterized by silencing of this gene. Interestingly, the expression of other DNA repair genes such as MRE11, MBD4 that are known targets of the mutator phenotype significantly discriminated GC on the basis of their MSI status. We also report for the first time that the SMUG1 uracil DNA glycosylase is differentially expressed in MSI versus MSS tumors. Besides MMR, this DNA glycosylase plays an important role in the repair of 5-fluorouracil (5-FU) that is one of the most important chemotherapeutic for sporadic colon and gastric cancer. The inactivation of MMR and BER (*via* inactivation of MLH1 and SMUG1) as seen in our MSI tumors gives a possible mechanistic basis to the observation that patients with MMR defective tumors are relatively resistant to 5-FU treatment. In summary, we have shown that GC with MLH1 silencing presents a characteristic transcriptional profile where the immune response and

an efficient p53 gene response are the likely mechanistic basis for long-term survival. The translation of the DNA repair gene expression profile of these tumors to clinical practice remains a challenging objective that should be pursued.

– Characterization of the functional role of DNA polymerase β splicing variants in human cancer
The frequent occurrence of DNA polymerase β. Pol β. gene variants has been described in tumor tissues but their role is still largely unknown. In this study a search for Pol β mutants and splice variants was conducted in matched normal and tumor gastric mucosa of patients suffering from gastric carcinoma, in human gastric cell lines and in gastric mucosa and blood samples from healthy individuals. Neither somatic nor hereditary mutations were found while a variety of alternative Pol β splicing variants were detected with high frequency in all the specimens analysed. The Pol β variant lacking exon 2 (Ex2Δ) and the isoforms with exon 11 skipping were detected in both normal and tumor tissues. The most frequent Ex2Δ variant was further characterized. We clearly demonstrated that Ex2Δ mRNA does not encode protein, as detected by both western blotting and immunofluorescence analysis of human gastric AGS cells, transfected with Ex2Δ variant. The absence of a functional protein was confirmed by comparing the DNA gap-filling capacity and methyl methanesulphonate sensitivity of wild type and Pol β null murine fibroblasts expressing the human Ex2Δ variant. We showed that the Ex2Δ transcript is polyadenylated as detected by PCR and its half-life is significantly longer than that of the wild type mRNA as inferred by treating AGS cells with actinomycin D. Moreover, we found that it localizes to polyribosomes that is compatible with a role as post-transcriptional gene regulator. This study identifies a new type of DNA repair variants that do not give rise to functional proteins but to non coding RNAs that could either modulate target mRNAs or represent unproductive splicing events. The collaboration with the USA partner is testified by the co-supervision of the doctoral thesis of Palma Nieves who has spent two years in Dr Wilson's laboratory and by the joint publication of the two laboratories that has been recently submitted for publication (Simonelli *et al*., submitted).

Publications of the project

D'Errico M, de Rinaldis E, Blasi MF, Viti V, Falchetti M, Calcagnile A, Sera F, Saieva C, Ottini L, Palli D, Palombo F, Giuliani A, Dogliotti E. Genome-wide expression profile of sporadic gastric cancers with microsatellite instability. *Eur J of Cancer* 2009;45(3):461-9.

Simonelli V, Palli D, Wilson SH, Dogliotti E. The most frequent splicing variant of DNA polymerase β lacking exon 2 is a non coding RNA. *FASEB J* Submitted.