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Poster Communication Abstract – 6B.08

Fra14A2, THE MURINE ORTHOLOGUE OF COMMON FRAGILE SITE *FRA3B*, IS UNSTABLE *IN VIVO* IN SOMATIC AND GERM CELLS

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Common fragile sites (CFS) are large sequences prone to chromosome breakage, which are hotspots for chromosome rearrangements and colocalise to genomic alterations found in tumours (Durkin & Glover, Annu. Rev. Genet., 41:169-192, 2007). CFS may harbour genes whose mutation is cause of human disease. For example, *PARK2* at *FRA6E* is involved in a form of autosomal recessive juvenile parkinsonism. Breakages at CFS appear *in vitro* after replication stress, and one key question is whether this loci may be considered stable in human subjects under physiological conditions. *FRA3B* (3p14.2) is the most active CFS of the human genome and the tumour-suppressor gene *FHIT* represents its core of instability (Durkin & Glover, Annu. Rev. Genet., 41:169-192, 2007). Sequence conservation between human and murine CFS has been demonstrated (Helmrich et al, Genome Res., 16:1222-1230, 2006); *Fra14A2/Fhit* is the murine orthologue of *FRA3B/FHIT* (Glover et al., Cancer Res., 58:3409-3414, 1998).

Molecular combing is a single molecule approach allowing a fine description of the structural and replication features of chromosomal regions (Palumbo et al., Chromosoma, 119:575-87, 2010). By molecular combing we have found that in normal human cells a small but relevant percentage of sequence abnormalities is present within the core of instability encompassing exon 5 of *FHIT* at *FRA3B*. Interestingly, the region is coincident with that described in cancer (Durkin & Glover, Annu. Rev. Genet., 41:169-192, 2007).

Aim of the study is to evaluate the *in vivo* stability of CFS *Fra14A2,* the murine orthologue of *FRA3B*, in the absence of exogenous replication stress. We are particularly interested in describing the modality of expression of the fragile site in the mouse germ cells, in order to evaluate if these genomic regions maintain the unstable features described in culture, and if they contribute to a genetic risk for the progeny. In mouse embryonic fibroblasts (MEF), used as controls, *Fra14A2* appears to be very active in response to aphidicolin-induced replication stress. Moreover, according to molecular combing results, the untreated MEF cells are characterised by spontaneous genomic instability occurring within *Fhit*. In particular, by using a probe pair identifying the orthologous sequence of the human core of instability, we found a rather heterogeneous pattern of hybridization suggesting the occurrence of deletions and rearrangements within the region under study. This is in agreement with our data on human cells. Molecular combing has been applied also on elongated DNA from epididymal sperm of adult C57Bl/6J mice. Interestingly, sequence instability events, similar to those observed in culture in MEF cells and in human cell lines, were found. Therefore mature gametes may carry sequence abnormalities at *Fra14A2/Fhit*.

We also considered the replication profile of the murine fragile site. Whole genome replication parameters of MEF cells appeared comparable to those observed in human primary

fibroblasts. In particular, fork rates were 1.49±0.06 kb/min, inter-origin distances were 136.7±12.8 kb. The single locus data are in progress. As a further step we aim to evaluate the spontaneous instability of *Fra14A2* in different somatic compartments with diverse proliferation activity, to better understand the relationships between replication activity and instability of CFS.