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Gene amplification in human and rodent cell lines defective in the *BRCA2* gene

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Gene amplification (GA) is involved in tumor progression, being one of the mechanisms of activation of proto-oncogenes and one of the causes of resistance to the chemotherapy. This work is part of a large project aimed at testing whether the proteins involved in the DNA damage response play also a role in GA. The *BRCA2* gene is a cancer susceptibility gene that plays a direct role in the repair of DNA damage. We used two BRCA2 mutant cell lines: a human cell line (EUFA423/F) provided by Dr. S. Powell (Washington University), and a Chinese hamster cell line (VC8) provided by Dr. M. Zdzienicka (Leiden University). Both lines have biallelic mutations in *BRCA2* and express truncated protein.

Thanks to the collaboration with Prof. A. Faucitano (LENA, Pavia), using a ^{60}Co γ-ray source, we showed that these two cell lines are hypersensitive to γ-irradiation. To test whether the BRCA2 defect affects GA we measured the frequency and rate of occurrence of PALA (N-Phosphonacetyl-L-aspartate) resistant colonies in these cell lines. In fact, the PALA resistance is mainly due to amplification of the CAD gene, that encodes for the trifunzionale protein CAD involved in pyrimidine biosynthesis. Our results show that in both BRCA2-deficient cell lines the frequency of PALA resistant colonies and the rate of occurrence of PALA resistant colonies are 2-12 times higher that in the control lines. We then analyzed by FISH (fluorescent *in situ* hybridization) the organization of the *CAD* gene in several PALA resistant clones and confirmed that GA is present in most of them. In conclusion, a defect in BRCA2 makes both human and hamster cells more prone to GA. These results are in agreement with our previous observation that impairment in DNA double strand break repair facilitates GA (2), supporting the hypothesis that DNA amplification is the result of an aberrant repair pathway.

References:

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