GENOTOXICITY BIOMARKERS MICRONUCLEUS, NUCLEOPLASMIC BRIDGES AND NUCLEAR BUDS

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INTRODUCTION

The increase of mortality from cancer brought urgency in identification and validation of predictive markers of risk and therefore early diagnosis. There is evidence that cytogenetic biomarkers are positively correlated with risk of cancer, and this is validated by studies of cohort and case-control. Cytokinesis-blocked micronucleus (CBMN) assay is used extensively in molecular epidemiology, and can be considered as a "cytome" assay covering cell proliferation, apoptosis, necrosis and chromosomal changes.
The chromosomal alterations most reported and studied by the CBMN are: micronucleus (MN), nucleoplasmic bridges (NPB) and nuclear buds (NBUDS).
The use of the MN assay in biomonitoring studies had a large increase in the last 15 years and international projects such as the HUMN (http://www.humn.org) have helped to increase the applicability and reliability of these tests.

AIM OF THE STUDY

These work pretend to show the importance of the CBMN assay and the importance of MN, NPB and NBUDs as biomarkers of genotoxicity.

REVIEW

MICRONUCLEUS

MN are classified as biomarkers of breakage and loss of chromosomes. They are small, extranuclear bodies that arise in dividing cells from acentric chromosome/chromatid fragments or whole chromosomes/chromatids that lag behind in anaphase and are not included in the daughter nuclei in telophase. The combination of the MN assay and FISH with probes labeling the pan (peri-) centromeric region of chromosomes provides the methodology to distinguish between micronuclei containg a whole chromosome (C+MN) and an acentric chromosome fragment (C-MN). The assay either provides the ability to detect both clastogenic and aneugenic events (Fig. 1).

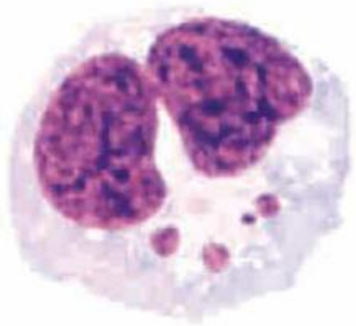

Fig. 1 - MNs in a lymphocyte

NUCLEOPLASMIC BRIDGES

NPB were validated by a study of Umegaki & Fenech (2000) as a biomarker of DNA damage in human WIL2-NS cells treated with hydrogen peroxide, superoxide or after co-incubation with activated human neutrophils.
NPB have their origin in dicentric chromosomes in which the centromes have been pulled to the opposite poles of the cell at anaphase and are therefore indicative of DNA mis-repair, chromosome rearrangement or telomere end-fusions. NPBs can break and origin MN. In 40% of the cases, 2 or more MN are formed by breakage of at least one bridge. The importance of scoring NPBs should not be underestimated because it provides direct evidence of genome damage resulting from mis-repaired DNA breaks which is otherwise not possible to deduce by scoring MN only which could originate from acentric fragments or chromosome loss (Fig.2).

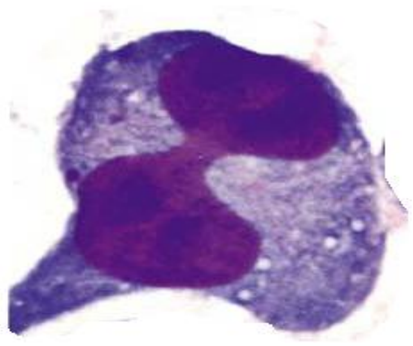

Fig. 2 - NBP in a lymphocyte

NUCLEAR BUDS

Over the past decade another unique mechanism of micronucleus formation, known as nuclear budding has emerged. The nuclear budding process is the mechanism in which cells remove amplified and/or excess DNA and is therefore marker of gene amplification and/or altered gene dosage. Shimizu et al. (1998) showed that amplified DNA is localised selectively to specific sites at the periphery of the nucleus and eliminated via nuclear budding to form MN during the S phase of cell cycle (Fig. 3).

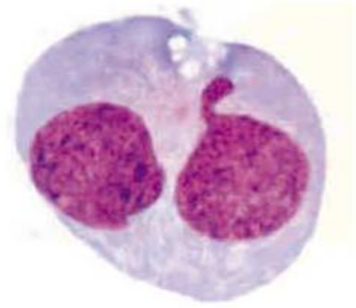

Fig. 3 - NBUD in a lymphocyte

SUMMARY

CBMN is a genotoxicity assay that allows simultaneous information about chromosomal damage resulting from loss of chromosomes, chromosomal rearrangements and gene amplification. Compared to other cytogenetic assays, the quantification of MN, NPB and NBUD provides several advantages, including speed and easy of analysis and it is not necessary cells in metaphase. The fact of the analysis is made in cells that had divided once is in latter assumption, prevention of confounding factors caused by differences in the kinetics of cell division.

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