CURRENT METHODS TO ASSESS GENOTOXICITY

Genotoxins are chemicals or drugs or any entities that cause damage to chromosomes, DNA or RNA. The damage can result in mutations, single or double stranded DNA breaks and impaired transcription and translation. If the damage occurs in somatic cells, the consequences can include the development of tumors, cell death and inflammation but if the damage occurs in germ cells, it can cause heritable diseases, reproductive issues and birth defects. Drugs with genotoxic potential cause damage that may or may not be repaired by cellular mechanisms so if the repair mechanisms are not able to adequately repair the damage, mutations are generated that may have disease causing potential.

Due to the significant potential impact of genotoxic damage, it is critical to test new therapies for genotoxic stress potential. Since the endpoints of genotoxic testing are defined, several relatively simple bacterial and mammalian cell models are available[1]. One of the earliest genotoxic tests was the bacterial Ames assay which assesses genotoxic potential by measuring mutations in specific strains of Salmonella bacteria that carry a mutation in the gene required to synthesize the amino acid histidine. The bacteria are cultured in media containing histidine and then exposed to the candidate drugs. The mutagenic potential of drugs is evaluated by determining if they cause reverse mutations and allow the bacteria to metabolize histidine in the culture and the number of bacterial colonies is a gauge of high, medium or low mutagenic potential[2].

Currently, two assays are popularly used to assess genotoxic stress – the Comet assay and the Micronucleus assay. The Comet assay uses single-cell gel electrophoresis assay to assess genotoxicity. The assay principle measures single- or double-stranded DNA breaks caused by drugs as cleaved DNA fragments migrate out of the cell when current is applied (ie. electrophoresis) while the undamaged DNA remains in the cell and forms the head of the comet. The denatured undamaged and cleaved DNA are stained with a DNA intercalating dye and visualized using fluorescence. While the Comet assay is simple and rapid and can be run on almost any eukaryotic cell, it does not shed any light on the mechanism of genotoxicity. The micronucleus test is also widely used to assess genotoxicity as micronuclei are essentially extra-nuclear bodies that include damaged chromosome fragments that result from chromosomal aberrations or genotoxic stress of specific drugs[3]. The chromosomal fragments from the micronuclei are not included in the nucleus after mitosis or meiosis so the genotoxic potential of drugs can be determined by counting the number of micronuclei. In many cases, the Comet assay and Micronucleus assay are both performed to assess the potential of drugs to cause DNA damage as well as chromosomal aberrations[4]. An interesting study from 2013 compared the Comet assay and Micronucleus for sensitivity and found that the Comet assay required higher doses of the test drugs and is less sensitive[4]. Nevertheless, both assay types provide valuable data on genotoxic stress. Research into the underlying mechanisms of genotoxicity is limited but some work has been done on drugs such as dacarbazine that is a chemotherapeutic approved to treat melanoma and Hodgkin's lymphoma[5]. Dacarbazine is known to cause DNA methylation that impact transcription and translation.

At this time, the field is focused on using these assays to determine if specific chemicals, drugs or environmental toxins can cause DNA damage using simple endpoints but it is likely that more complex assays using next-generation sequencing will be broadly adopted to assess genome-wide genotoxic stress and understand mechanisms and hotspots for DNA damage[6].

References:
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