

Mutagenesis

Introduction

Mutagenesis or also *genotoxicity* is a process in which *DNA* is damaged by the action of *mutagenic factors* (*mutagens*). This term is often associated with carcinogenesis, *which is a process in which the influence of carcinogens causes DNA damage*, resulting in the transformation of a normal cell into tumor cell. These terms cannot be interchangeably used. It applies here that every carcinogen is a mutagen, but not every mutagen has been proven to have carcinogenic activity. This article should contribute to the understanding of the basic principles of mutagenesis, giving a brief overview of the basic types of mutagens and the basic types of genotoxicity testing. It is aimed generally, practically, only some of the most common and model examples are always given. Therefore, it is intended for undergraduate medical students and the lay public.

Mutagenic Factors

Mutagenic factors are a diverse group.

1. According to their nature and essence, we divide them into physical, chemical and biological mutagens.

- **Chemical mutagens** cause mutations based on chemical modification of the *nucleotide* (mainly bases) and changes in the polynucleotide chain (cleavage, spacing, etc.). Here are a few examples to give you an idea. **Alkylating, arylating substances** (yperit, benzo(a)pyrene) form so-called "adducts" with nucleic acid bases. Furthermore, there are so-called **intercalates** - e.g., fluorescent dyes or **other chemical substances** causing deamination, hydroxylation, cleavage of bonds in DNA, RNA. The various **synthetic base analogs** after incorporation mainly cause substitution mutations because they mispair with the **complementary bases** during replication. Typical representatives from everyday life are various food dyes, combustion products (i.e. smoking and car exhaust) and PCB - components of plastic materials.
- **Physical mutagens** are mainly **ionizing radiation** and **UV radiation**. The effect of UV radiation causes mutations during replication - among the most common are pyrimidine dimers. *We are protected from this radiation by skin organs where these mutations are collected. In case of excessive exposure to UV light or in the case of malfunctions of the replication repair mechanisms,* a high accumulation of mutations can have a carcinogenic effect and result in skin cancers. *One of the most well-known genetically determined disorders, where even the repair of thymine dimers created after UV radiation is damaged, is xeroderma pigmentosum.*
- **Biological mutagens** are biologically active particles such as **viruses** (DNA, RNA, retro) and **transposons** (*Line, Alu, Sine*).

2. We further divide mutagens from a biochemical point of view, according to the mechanism of action, into direct and promutagenic substances.

- Direct mutagens are factors capable of causing some changes in DNA by their own nature and composition.
- Promutagens are harmless in themselves, they become mutagenic only after metabolic activation, which enables their processing by the organism.

Genotoxicity tests

In order to find out which substances and factors are to what extent mutagenic and dangerous to humans, so-called mutagenicity or genotoxicity tests are carried out. Basic laboratory testing is performed at three levels - molecular, genetic and chromosomal.

1. **Tests at the molecular level** detect changes at the DNA level by, for example, the following mechanisms:
 - **determination of adducts**, immunohistochemical methods determine the degree of presence of *adducts* in the DNA test sample;
 - using **PCR** (polymerase chain reaction) and other molecular-biological methods, we determine the **occurrence of specific mutations**;
 - the so-called **unplanned DNA synthesis (UDS)** is based on the measurement of the rate of repair mechanisms (*repair syntheses*) proportional to DNA damage through incorporation a radiolabeled *nucleotide*;
 - the most famous so-called "**comet assay**" (**comet assay**) or '**SSGE (single cell electrophoresis)**' makes use of the fact that, due to mutagenic effects, fragments are formed in the nuclei of cells. Their quantity is proportional to the degree of genotoxicity of the tested potential mutagen on cell. We transfer the cell nuclei into a gel and using *electrophoresis* the resulting fragments are *released*. After staining the nuclei with a ``fluorescent dye and observing through a fluorescence microscope, we observe a formation resembling a flying comet, *whose body corresponds to the nucleus and the size (length and extent) of the tail corresponds to the number of DNA fragments, and thus also the degree of genotoxicity.*
2. **Gene-level tests** detect more extensive damage to entire genes. Gene mutations can be detected by the following biochemical methods:
 - **SOS/umu test** measures, similarly to unplanned DNA synthesis, the level of repair mechanisms that will take place on a substance that has been exposed to a mutagen, but now it concerns repairs within entire genes. Special strains of bacteria are used here and the level of expression of mutator genes (i.e. genes whose products are fundamentally involved in repair processes) is monitored.
 - **induction of resistant mutants** in mammalian cells in vitro, when we can induce the formation of mutants through the action of mutagens, which will be resistant to certain normally toxic chemical substances such as ouabain, 6-thioguanine, 8-azaguanine etc...
 - **Ames test**, one of the most widely used genotoxicity tests, allows to monitor reverse mutations in histidine-deficient strains of *Salmonella typhimurium*. These strains survive only in medium containing histidine. The action of mutagens in these bacteria causes a reverse mutation, which reverses the damaged gene and thus the bacteria regain the ability to produce histidine, which they previously lacked. The amount of living bacteria on a medium without histidine corresponds to the strength and quantity of the mutagen. The test is performed on three Petri dishes with mutant strains of bacteria in a medium poor in histidine. The dish to which no mutagen was added serves as a negative control and only a small amount of adapted viable bacteria appears here - so-called spontaneous revertants. In addition, the tested substance (potential mutagen) is added to the second dish, and proof of mutagenicity corresponds to a large number of viable revertants. The third dish serves as a positive control, and instead of the tested substance we add an already known mutagen, the effect of which is the same as in the previous case.
3. **Chromosome-level tests** detect a number of changes within *chromosome aberrations*, whether large or submicroscopic in scope. Mostly, it is damage to multiple genes, gene groups due to structural or numerical aberrations resulting from mutagenic action. The three most used methods include:
 - **Testing by detecting and determining the frequency of structural and numerical chromosomal aberrations** in mammalian cells in culture, in laboratory animals or in persons exposed to the tested mutagen. Due to this, e.g. monitoring of workers in risky workplaces is carried out. The number of aberrations in individual cells can be determined for each person using, for example, karyotyping, *CGH* or chip methods (array CGH). *Mutagenicity is further evaluated according to the value of the frequency of cells with a large number of aberrations, while a frequency of up to 2% is considered a normal finding, values of*

2-4% are borderline and a finding of 4% is considered to be *result of mutagen action*. It should be noted that with testing carried out in this way, we will reach an objective result if we test a sufficient number of people (approx. 20), preferably with a 50% representation of men and women

- Another option is to test sister chromatid exchanges (SCE - Sister Chromatid Exchanges). We monitor the rate - the sister chromatid exchange rate - *that increases in cells exposed to the mutagen. For detection, we can use dyeing with dye probes M-FISH or the SKY method, where the stained chromosomes are observed under a fluorescent microscope and the results are processed and recorded by a computer.*
- **Micronucleus test** or micronucleus formation test is based on the fact that the action of mutagens causes the formation of chromosome fragments without centromeres. These "acentric chromosomes" are not taken into the nucleus during mitosis and form so-called micronuclei in the cytoplasm. The rate of occurrence of micronuclei also informs us, like the previous tests, about whether the substance has a mutagenic effect and about the strength of the mutagen.

Links

External links

- [1] (<https://dl1.cuni.cz/>), medical biology course II.
- gzs1L6lv_yPhWM:&imgrefurl=http://www.ueb.cas.cz/cs/internet-site/pro-verejnost-novinare/roslina-s-pribehem%3Fpage%3D2&docid=-VhPU1gHEcn-xM&imgurl=http://www.ueb.cas.cz/cs/system/files/users/public/kolar_27/obrazky_popularizace/fotopribeh/fotopribeh_0410_mini.jpg&w=150&h=150&ei=SYIDT_HPGY-XOpWzhfcO&zoom=1 Institute of Experimental Botany AS CR - A plant with a story (<http://www.google.cz/imgres?q=kometov%C3%B4+test&hl=cs&client=firefox-a&hs=a&sa=X&rls=org.mozilla:cs:official&biw=1280&bih=579&tbnm=isch&prmd=imvns&tbnid=>)

Related Articles

- *mutagens and mutagenesis*

Sources

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- STROLLER, Eduard – NOVOTNÁ, Drahuše, et al. *Clinical Cytogenetics I : Introduction to clinical cytogenetics, investigative methods in clinical cytogenetics*. 2. edition. Prague : Karolinum, 2010. 134 pp. ISBN 978-80-246-1880-7.
- GOETZ, Paul, et al. *Selected Chapters in Medical Biology, Volume 2*. 1. edition. Prague : Karolinum, 2002. 139 pp. ISBN 80-246-0320-9.

<https://www.wikiskripta.eu/w/Mutageneze>

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