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Використання експериментів з мікроядрами для виявлення ризиків раку людини: короткий огляд

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Вступ. Мікроядра (МЯ) — це невеликі позаядерні ДНК-вмісні структури, які утворюються внаслідок структурних і числових хромосомних аберацій. Експерименти з МЯ у порівнянні зі звичайними хромосомними аналізами в метафазних клітинах є швидшими у виконанні та менш ресурсоємними. На сьогоднішній день, дослідження МЯ широко використовуються для рутинного скринінгу хімічних речовин *in vitro* та *in vivo*, а також для контролю навколишнього середовища та біомоніторингу людини.

Мета. Зібрати наукову інформацію щодо використання дослідження МЯ для виявлення підвищеного ризику раку внаслідок впливу чинників навколишнього середовища, способу життя та професійних шкідливостей, а також для виявлення/діагностики різних форм раку.

Методи. Аналіз літератури щодо методів дослідження МЯ в людей; а також використання цієї методики суміжних наукови галузях.

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Дозвіл комісії з біоетики щодо проведення досліджень: для цього дослідження непотрібний.

Фінансування: Українсько-австрійський білатеральний проект «Дослідження фізіологічних та біохімічних параметрів для встановлення простих, надійних та відповідних біомаркерів для лікувальних інтервенцій для пацієнтів з пост-травматичним стресовим розладом (ПТСР)».

Результати. На сьогоднішній день розроблено широкий спектр протоколів біомоніторингових досліджень для оцінки/вимірювання утворення МЯ у лювських клітинах, зокрема периферичної крові та епітеліальних клітинах різних органів (щічна та носова порожнини, шийка матки та сечовий міхур). На додаток до МЯ можна оцінити й інші ядерні аномалії, які відображають генетичну нестабільність, а також гостру токсичність і поділ клітин-мішеней.

Висновки. Зростає кіькість наукових доказів того, що МЯ мають діагностичну цінність як біомаркер підвищеного ризику новоутворів, зокрема для ранньої діагностики раку шийки матки та сечового міхура.

Ключові слова: мікроядра, людина, спосіб життя, харчування, професіні хвороби, рак.

Use of micronucleus experiments for the detection of human cancer risks: A brief overview

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Introduction. Micronuclei (MN) are small extranuclear DNA-containing structures that are formed as a consequence of structural and numerical chromosomal aberrations. The advantage of MN experiments compared to conventional chromosomal analyses in metaphase cells is that the scoring is by far less time consuming and laborious. MN experiments are currently widely used for the routine screening of chemicals *in vitro* and *in vivo* but also for environmental control and human biomonitoring

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Objectives. The purpose of this review was to collect data on the use of MN experiments for the detection of increased cancer risks as a consequence of environmental, lifestyle and occupational exposures and the detection/diagnosis of different forms of cancer.

Methods. Analysis of the literature on methods for MN experiments with humans; as well as the use of this technique in different areas of research.

Results. To date, a wide range of protocols for human biomonitoring studies has been developed for the measurement of MN formation in peripheral blood cells and in epithelial from different organs (buccal and nasal cavity, cervix and bladder). In addition to MN, other nuclear anomalies can be scored which reflect genetic instability as well as acute toxicity and the division of target cells.

Conclusions. The evidence is accumulating that MN can be used as a diagnostic tool for the detection of increased cancer risks as well as for the early diagnosis of cervical and bladder cancer.

Keywords: micronucleus, human, lifestyle, nutrition, occupation, cancer.

Introduction

Micronuclei (MN) are extranuclear DNA-containing bodies that are formed as a consequence of structural and numerical chromosomal aberrations (clastogenic and aneugenic effects). They were first described in the 1970s by the American cytogeneticist Heddle [1] and the Swiss cytogeneticists Matter and Schmid [2] and can be used to detect DNA-damaging effects in a variety of cell types and intact organisms, including plants, mollusks, fishes, amphibians mammals and humans (for review see [3, 4]). The advantage of MN experiments compared to conventional chromosomal analyses in metaphase cells is that the scoring is by far less time consuming and laborious. MN experiments are currently widely used for the routine screening of chemicals *in vitro* and *in vivo* but also for environmental control and human biomonitoring [5]. This article gives a brief overview on the use of MN experiments for the detection of increased cancer risks as a consequence of environmental lifestyle and occupational exposures and for the detection/diagnosis of different forms of cancer. fig. 1 schematically shows the causes of MN formation.

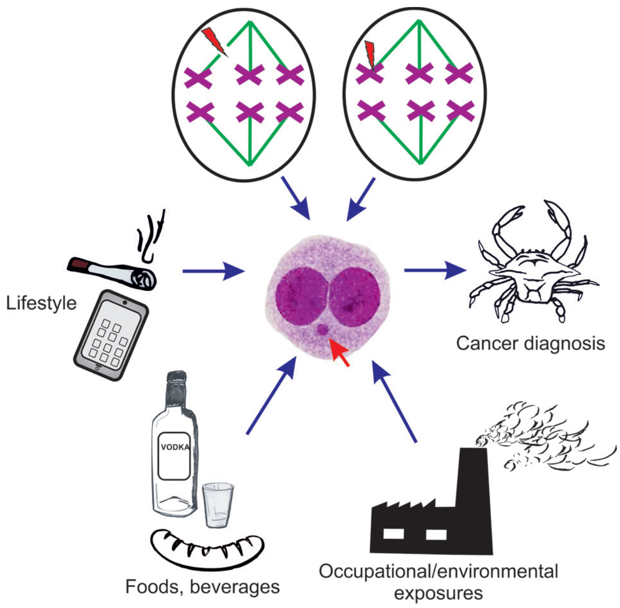

Figure 1. Micronuclei are formed as a consequence of numerical and structural chromosomal aberrations (left part: aneuploidy; right part: clastogenic effect). They can detect adverse health effects due to different exposures and, furthermore, they can be also used for the detection of several forms of cancer

Protocols for experiments with different cell types

MN studies with human cells can be either performed with lymphocytes which are collected from blood samples or with exfoliated cells from different organs. Figure 2 gives a schematic overview of different tests which can be performed with humans. MN were scored initially in mononucleated peripheral blood cells. A substantial improvement was achieved by M. Fenech who developed the MN assay in the early 1980s [6]. This procedure is based on the use of cytochalasin B which allows the scoring of MN in binucleated cells. MN formation requires nuclear division and the use of cytochalasin B has the advantage that it can be used to identify cells in which the nuclei are divided.

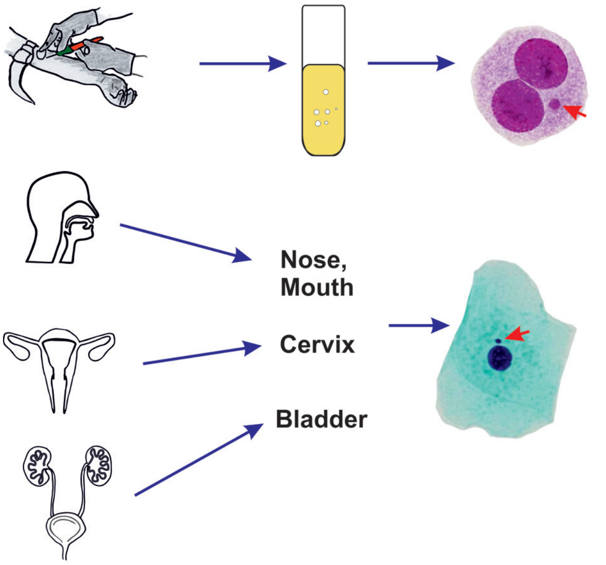

Figure 2. A schematic overview of different tests, which can be performed with human cells (lymphocytes and different types of epithelial cells)

Apart from MN, a number of other anomalies can be scored in lymphocytes which reflect genetic damage (nuclear bridges reflecting the formation of dicentric chromosomes and nuclear buds reflecting gene amplification) as well as cytotoxicity (apoptosis, necrosis). Furthermore, it is possible to determine the mitotic activity of cells which reflect the integrity of the immune system [7]. A comprehensive description of the procedure can be found in a publication of Fenech [8], picture galleries showing the morphology of different anomalies have been published by Fenech et al. [9].

Exfoliated cells can be collected from various tissues. The most widely used approach is MN experiments with cells from the oral mucosa. Apart from MN, other anomalies can be scored, for example, nuclear buds and so-called "broken eggs", an anomaly that possibly reflects gene amplification, as well as binucleates that reflect the failure of cytokinesis [10]. Acute cytotoxic effects lead to karyorrhectic, karyolitic and pyknotic cells and cells with condensed chromatin [11]. The standard protocol is described in a paper by Thomas et al. [12]. Pictures of different anomalies can be found in articles by Bolonesi et al. [13].

Cells from the oral cavity can be collected easily using spatulas and toothbrushes. For individuals who are exposed to chemicals via inhalation, it is also possible to collect cells from the nasal mucosa using cytobrushes. Nuclear anomalies which can be evaluated in these cells are identical to those which are scored in buccal cells. A comprehensive review on the use of nasal cells was recently published by Knasmueller et al. [14]

Cells from the bladder are sampled from the urine (by centrifugation). They were mainly used for MN screening but also other anomalies can be evaluated [15]. About 75 individual studies have been published so far; they concern the detection of effects caused by chemical exposures but also the diagnosis of bladder cancer [16].

Cervical cells were mainly scored in the course of routine Pap tests. The morphology of nuclear anomalies is similar to that of oral mucosa cells; a comprehensive review on the use of this test system and the methodology has been published by Setayesh et al. [16, 17].

Use of different techniques

The most frequently used models are experiments with blood cells, followed by MN studies with buccal cells. Figure 3 gives an overview of the number of studies that have been published in the scientific literature so far.

Prediction of human cancer risks

Bonassi et al. [18, 19] analyzed associations between MN formation in lymphocytes and human cancer in a number of studies. They found that MN, as well as chromosomal aberrations, are reliable biomarkers for the detection of increased cancer risks [20, 21].

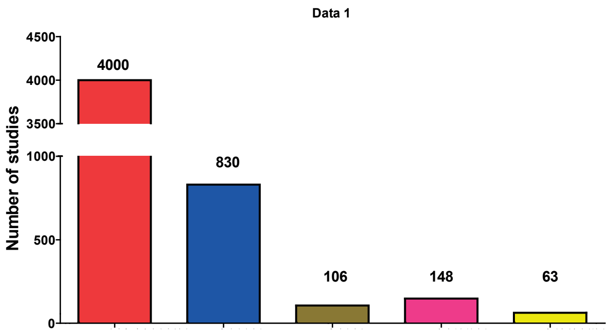

Figure 3. A number of MN studies have been published with peripheral blood cells and cells from different organs. The numbers were calculated based on comprehensive computer-aided literature searches in scientific databases (Google Scholar, PubMed, Scopus)

Ceppi et al. [22] compared MN formation in buccal cells and lymphocytes and found a high correlation indicating that MN rates in oral cells can be also used for the detection of increased cancer rates. Furthermore, it is notable that it was reported in some studies that individuals with pre-cancerous lesions in the mouth (e.g. leukoplakia and dysplasia) have increased MN rates [23]. It was also found that exposure to carcinogenic chemicals at workplaces led to MN formation in nasal and oral cells [14, 24]. Only a few investigations concerned the impact of chemical carcinogens on MN formation in urothelial and cervical cells, diseases and infections which are associated with increased cancer risk (e.g., schistosomiasis, human papillomavirus, HPV), lead to elevated MN rates (for review, see [16, 17]).

Examples for effects of occupational exposures

Numerous MN studies have been realized with lymphocytes and buccal cells of exposed workers. Recent studies with blood cells are described in a special issue in the review section of Mutation Research [25]. Examples of such studies are listed in Table 1.

Lifestyle-related effects

A number of studies have been developed, which concern the induction of MN as a consequence of smoking and chewing different types of plant material (khat, coca, betel, and tobacco; for review, see [50]). Positive results were obtained in some studies with blood cells and also with oral mucosal cells. However, the effects of tobacco smoking in lymphocytes are only moderate [51]. In buccal cells, we found that MN ratios increased in heavy smokers with the tar content of cigarettes, while a reverse association was observed with the nicotine concentration [52].

Smoking also leads to MN formation in the bladder [53] and cervix cells [54].

Investigations concerning the impact of the use of mobile phones led to conflicting findings in lymphocytes [55, 56] and buccal cells [57, 58].

Table 1

Examples of occupational exposure, which causes micronucleus formation in lymphocytes or epithelial cells (buccal, nasal, urothelial)

Occupation	Exposure	Reference
Carpenters	Wood dust, formaldehyde	[26]
Electroplaters, welders	Heavy metals, acids, fumes and gases	[27, 28]
Road markers	Silica particles, paints, number of organic chemicals (butyl- and ethyl-acetate, butyl acrylate, heptane, methylcyclohexane, methyl hexane, methyl methacrylate and benzoyl peroxide	[29]
Painters	Paints, lead, acetone, toluene, dichloromethane	[30, 31]
Tannery workers	Chromium, aniline, formaldehyde, acids (formic and sulfuric), solvents (benzene, ethanol, tetrachloride, trichloroethylene, dichloromethane)	[32, 33]
Medical staff	Cytostatics, ionizing radiation, anesthetic gases, ethylene oxide	[34, 35]
Textile workers	Dyes, formaldehyde, arsenic, cadmium, cotton dust	[36, 37]
Miners	Radon, dust, methane, coal dust	[38, 39]
Fuel station attendants	Fuel, organic solvents, gasoline vapors	[40-42]
Construction workers	Asbestos, dust, silica	[43, 44]
Agricultural workers	Pesticides, herbicides, fertilizers, plant growth hormones	[45, 46]
Asphalt workers	Polycyclic aromatic hydrocarbons, solvents, fumes, aerosols	[47-49]

Impact of nutritional factors on MN frequencies

It was postulated in a number of studies that micronutrients, for example, calcium, folate, nicotinic acid, vitamin E, retinol, beta-carotene, pantothenic acid, biotin and riboflavin have an impact on the stability of genetic material [59].

Most studies concerned the effects of folate and it was shown that deficiency leads to increased formation of MN in peripheral blood cells, while supplementation reduced MN rates in individuals with low blood levels. Furthermore, it was found that vitamins B6 and B12 play a crucial role [60] These vitamins are required for the synthesis of DNA bases and it is known that deficiency leads to misincorporation of uracil instead of thymidine and as a consequence to DNA instability, chromosomal aberrations and MN formation [60].

Another relevant factor is hypercaloric nutrition. It was shown in a number of human studies that overweight leads to increased MN rates, while reduction of body weight in obese persons led to stabilization of the genetic material [61]. Molecular mechanisms for the impact of increased body fat on genetic stability are inflammatory responses and the release of reactive oxygen radicals. A detailed overview can be found in the article by Setayesh et al. [61].

Micronucleus formation and diseases

As mentioned above, it is known that MN in lymphocytes is a reliable biomarker for increased cancer risk in humans. In a more recent study, it was shown that MN rates in blood cells can also predict the risk of lung cancer in smokers [62].

MN in urothelial cells is indicative of bladder cancer and may be useful as a diagnostic tool and for the surveillance of this frequent form of cancer [63]. A recent meta-analysis by Setayesh et al. [17] showed that MN rates in cervical cells increase with the degree of malignancy of neoplastic lesions. Based on these findings it was postulated that MN scoring could improve the diagnosis of cervical cancer [64].

It is also notable that a number of studies indicated that NM rates may be predictive for the success of radiation therapy. Individuals which are responsive to the therapy are more sensitive in regard to induction of chromosomal damage, which leads to the elimination of cancer cells [65, 66].

A recent special issue of Mutation Research concerns associations between MN frequencies and various diseases. It is notable that it was found in a number of studies that neurological diseases (Parkinson, Alzheimer), as well as autoimmune disorders, chronic inflammation and diabetes, are characterized by elevated rates of MN (for details see [67]).

Conclusions and outlook

Micronucleus experiments with lymphocytes and buccal cells are now the most widely used approaches in occupational monitoring [68]. They were also successfully used to assess the impact of nutritional and lifestyle factors on human health. Experiments with urothelial and cervical cells can be used to detect groups of individuals that have increased risks for bladder and cervical cancer and possibly for the surveillance of patients. Many studies show that MN, which are formed as a consequence of structural and chromosomal aberrations are a reliable biomarker for the assessment of human cancer risks.

The additional evaluation of nuclear anomalies other than MN provides further valuable information about genotoxic effects and acute cytotoxicity caused by chemical exposures.

At present, occupational/environmental monitoring is primarily based on the measurement of exposure to individual hazardous chemicals. Biomonitoring studies have the advantage that they also reflect combined (synergistic and antagonistic) effects and provide information about the impact of demographic factors (e.g., age, body weight, gender) that may affect the sensitivity toward toxins. The development of automated scoring devices (which is described in several recent articles [69, 70]) will contribute to the reduction of the costs for routine screening of humans and should be combined in the future with chemical analytical measurements.

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