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Position statement of the ZKBS on safety measures for handling nucleic acids with neoplastic transformation potential

In 1991, safety measures were established in a position statement of the ZKBS in order to mitigate potential risks when working with so-called "nucleic acids with oncogenic potential". This was occasioned by studies in which the development of malignant tumors was observed in laboratory animals after oncogenic nucleic acid segments had been injected into these animals or applied to lesions in their skin. This resulted particularly from a transformation of epithelial cells [1-5].

The following measures were recommended to counteract a hazard to laboratory personnel handling these nucleic acids:

1. Disposable gloves should be worn when working with nucleic acids possessing the above-mentioned hazard potential.
2. The use of sharp, pointed or breakable laboratory equipment should be avoided, whenever possible.
3. Laboratory work sites and laboratory equipment coming in touch with these nucleic acids should be carefully cleaned after cessation of work.
4. Laboratory waste containing such nucleic acids should be denatured by autoclaving or applying chemical methods.
5. Persons with considerable skin injuries (open eczemas, wounds and infections) or with pronounced verrucosis (multiple wart formation) should not carry out any operations with these nucleic acids.

The recommendation of these additional personal safety measures remains unchanged.

However, the updated position statement determines new criteria for the evaluation of a human-relevant neoplastic transformation potential of nucleic acid segments, including noncoding RNAs or DNA segments:

A nucleic acid segment will be classified as a potential cause of neoplastic transformation in human cells if its

causal involvement in the development of tumors

has been proven based on

- an in vitro transformation of relevant vertebrate cells which enables their anchorage-independent growth,

  and/or

- the development of tumors in relevant animal models, especially in xenograft or direct vertebrate animal models (e.g. mice, rats, zebrafish).

Explanations

This position statement is exclusively concerned with cancerogenous nucleic acid segments whose transcription and/or expression alone suffices to induce a neoplastic transformation in a human cell after an accidental uptake. It is not concerned with such nucleic acid segments which

modulate or enhance, but do not induce, tumor progression. Examples for modulating processes are [7, 8, 9]:

(1) resistance to apoptosis signals

(2) maintenance of the cell-division signal and thus an increased cell-division rate and/or immortalization

(3) genomic instability

(4) stabilization of the telomeres

(5) supplying the transformed cells with nutrients via the blood circulation (angiogenesis)

(6) invasion into tissues foreign to the cell (metastasis)

(7) accompanying inflammation-mediating processes in the so-called microenvironment of a tumor

(8) conversion of the cellular metabolism from aerobic to anaerobic energy production

(9) evasion of recognition and destruction by the immune system.

It must be noted in this regard that one of these characteristics alone is not sufficient to transform a healthy cell into a cancer cell. Instead, for example, it has been shown that the immortalization of cells is only an initial step in neoplastic transformation. The immortalization of primary human cells in vitro by means of various cancerogenous substances has been often described. Morphological alterations of the cells which permit inferring tumorigenic properties, however, were only induced after a second impulse, for example, by means of an infection with retroviruses, an exposure to radiation or an incubation with chemical cancerogenous substances [6]. Immortalization in a cell-culture model resulting from the introduction of a nucleic acid segment can thus not be taken as the sole criterion for the classification of a nucleic acid segment as neoplastic transforming.

Instead, the identification of a neoplastic transformation potential requires evidence that the introduction of the nucleic acid segment into the cell causes contact-independent growth. If this evidence is obtained with the aid of cells or cell lines which were isolated or established from a tumorous tissue it must be assumed that one or several transformation-inducing signals had already been effective. Data derived from such experiments can therefore only to a limited extent give indications as to the efficacy of the latest introduced nucleic acid segment and cannot be decisive for evaluation. Decisive for the evaluation of human-relevant nucleic acid segments are data based on relevant primary cells or immortalized cells which are not cancer cells and have been isolated from vertebrates.

In research, a variety of animal models are used to study tumorigenic substances and/or develop and treat human tumors. As compared with other mammalian models, the mouse has the advantage that we possess many years of experience in how to introduce and study the effects of defined genetic alterations. Usually, by applying various techniques, cancer-mouse models are generated by introducing oncogenes into the genome of the entire animal or into certain tissues, or already existing oncogenes are activated and/or tumor suppression genes are inactivated [13].

Apart from the direct vertebrate animal models, xenograft transplantations provide a "human in the animal" model, in which human tumor cells are introduced into another species, so that human tumors will develop in these animals. The mouse model commonly uses immune-suppressed animals such as Nod-SCID strains, as the murine immune system in healthy animals especially attacks heterologous cells and would thus prevent the growth of a human tumor. However, studies with xenograft models in fish embryos (Danio rerio, Xiphophorus sp., Oryzias latipes) are being increasingly applied. Nowadays they constitute very well established systems for studying human skin tumors [10-12].

At this point, it needs to be said that the use of chemical or physical cancerogenous factors might impose an increased risk when concomitantly working with nucleic acids derived from oncogenes.

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