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Survival of aneuploid, micronucleated and/or polyploid cells: Crosstalk between ploidy control and apoptosis

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Abstract

Microtubule inhibitors are known to block the cell cycle at M-phase, by damaging the mitotic spindle. However, under certain circumstances, cells can escape these effects and become aneuploid, polyploid and/or micronucleated. It is well known that aneuploidy can have adverse effects on human health such as pregnancy wastage, birth defects and the development of human tumours. The present paper aims at reviewing the data our laboratory has accumulated during the last years about the relation between aneuploidy/polyploidy/presence of micronuclei and the induction of apoptosis in human cells after *in vitro* exposure to the microtubule inhibitor nocodazole. Exposure to high doses of nocodazole results in polyploidy due to mitotic slippage in the absence of a functional spindle. Depending on their p53-status polyploid cells may eventually arrest, die or continue cycling. In these experimental conditions, our data showed that polyploidy does not constitute a strong apoptotic signal. In case of exposure to low concentrations of nocodazole, microtubule depolymerization is disturbed resulting in a spindle with damaged microtubules. This can give rise to chromosome loss and non-disjunction. Our data showed that in particular micronucleated cells, originating from chromosome loss can be eliminated by apoptosis. In addition, nocodazole-induced apoptosis involves the apical caspase-8 and -9 and the effector caspase-3. We show evidence that caspase-3, in addition to its function in apoptosis, plays a role in the formation of micronuclei.

Keywords: Aneuploidy; Polyploidy; Apoptosis; Nocodazole

1. Introduction

It is well known that aneuploidy can have a severe impact on human health conditions. Aneuploidy in germ cells contributes to mental retardation, congenital malformations and pregnancy wastage in human beings and aneuploidy in somatic cells is involved in the development of human tumours [1]. Aneuploidy and polyploidy are often associated with malignant transformation.

The fact that individuals with particular aneuploidies (*e.g.* Down syndrome) can survive and that polyploidy is found as a normal condition in several organs, like the liver in humans [2], indicates that aneuploidy and polyploidy are in some situations also compatible with normal cell life. However, several reasons can also be found to consider that aneuploidy or polyploidy could trigger apoptosis: maintenance of karyotype stability is the aim of mitotic cell division and from a mechanistic point of view, it is expected that the induction of apoptosis can contribute to the elimination of cells with premutagenic lesions (which can still be repaired) or mutations [3]. Furthermore, several studies suggested the induction of apoptosis by nocodazole *in vitro* in mammalian cells [4,5].

Among chemicals inducing polyploidy and aneuploidy are the so-called spindle poisons, such as nocodazole, which interfere with the formation of the spindle. Many of these compounds are used as cytostatic chemotherapeutic agents or other pharmaceuticals, but also find their application as fungicides and antihelminthics. These drugs alter the polymerization dynamics of microtubules thereby blocking mitosis. As chemotherapeutic agents, microtubule inhibitors are used at high concentrations to block cell division and kill tumour cells. Due to their specific effects on cell division it is not surprising that these neoplastic drugs can also induce aneuploidy and polyploidy. Therefore besides their capability of defeating a primary tumour, they

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also carry the risk of inducing secondary tumours. Cytostatic chemotherapy agents are also used in immunotherapy [6], where they are applied at lower concentrations. This also implicates a possible risk for the induction of aneuploidy. As antihelminthics and fungicides they are applied in high concentrations to kill fungal pathogens or other harmful organisms. However, it should be taken into account that also exposure to low concentrations of these compounds can occur by dietary, environmental and occupational exposure. Considering the importance of microtubule inhibitors not only as chemotherapeutic agents, but also as fungicides and antihelminthics, it is of major concern to know their concentration-dependent effects on the ploidy status and the survival of cells with aberrant chromosome numbers. This knowledge would allow to improve their efficiency and to avoid harmful (secondary) effects.

Our aim is to give an overview of recent results obtained by our laboratory on the relation between aneuploidy/polyploidy/presence of micronuclei and the induction of apoptosis in human peripheral mononucleated cells (PBMC) and cell lines after *in vitro* exposure to low and high concentrations of the microtubule inhibitor nocodazole. Our working hypothesis was that the cell has sensors for accurate chromosome segregation during metaphase–anaphase transition and for euploidy in G_1 phase. If the corresponding checkpoints are not satisfied, the signals may trigger apoptosis.

For human risk assessment, the influence of cellular factors (apoptosis, metabolism, DNA-repair, defence against oxidative stress) affecting threshold values should be analyzed preferentially in human cells and, still better in primary cells, *e.g.* human PBMC. However, to understand particular mechanisms it is interesting to use specific paired cell lines presenting deficiencies versus (over) expression of the target gene function.

A major advantage of using nocodazole as a model microtubule-depolymerizing agent is its highly specific binding site, Arg 390 on β-tubulin [7]. This allows to study in detail the effects of its binding to tubulin, such as the number of binding sites, to calculate the number of events necessary to induce aneuploidy, and moreover, one can assume that there is no other target and that all effects observed are related to this specific interaction with microtubules. Nocodazole is often used for mechanistic studies of microtubule dynamics because of its reversible characteristic. However, this was not applicable in the protocols used in the present studies where cells were always continuously exposed to the compound.

2. Survival of aneuploid and polyploid cells

The survival of polyploid cells that were formed after exposure to a high concentration of nocodazole (0.303 μM) was investigated in human cells. Continuous exposure to this concentration of nocodazole leads to a significant decrease of microtubule depolymerization. In the absence of a functional spindle, cells can exit mitosis and progress to the following interphase without chromatid segregation, a process called mitotic slippage, yielding 4N, 4C cells. Exposure to a high concentration of nocodazole (0.303 μM) in the erythroleukemia cell lines K562 (not expressing *p53*) and KS (expressing *p53*) leads to the induction of apoptosis, confirming the observations in PBMC, that spindle inhibitors like nocodazole induce apoptosis [6]. Moreover, our data also demonstrated that apoptosis is induced independently of p53 in response to mitotic spindle failure, since apoptosis was observed in both cell lines [5].

Given the positive correlation between apoptosis, abnormal metaphases and polyploidy found in PBMC [8], the link between apoptosis and polyploidy was further investigated in K562 and KS cell lines. Since KS expresses the wildtype *p53* gene and K562 does not, the use of these cell lines allowed us also to investigate the possible role of p53 in the survival of polyploid cells. It has already been found that p53-negative cells become aneuploid or polyploid at a higher frequency when treated with spindle poisons [9–11], suggesting a role for p53 in the regulation of polyploid cell propagation via activation of a post-mitotic checkpoint [12]. Moreover, it was demonstrated by Casenghi et al. [5] that p53 is required for the control of ploidy in cells with an impaired mitotic division and that apoptosis is induced independently of p53 in response to mitotic spindle failure. To investigate the survival of polyploid cells FISH analysis with pericentromeric probes for chromosomes 1 and 17 was performed on apoptotic and viable cells, obtained by annexin-V staining combined with flow cytometry. Annexin-V staining is based on a reversal of cell membrane asymmetry and detects early apoptotic cells. Our data showed that in the p53-proficient KS cells, exposure to nocodazole induced a similar fraction of hexaploid cells in both viable and apoptotic cell fractions, but no dodecaploid cells were ever observed (Fig. 1). In the p53-deficient K562 cells on the contrary, a population of dodecaploid cells, which were essentially viable, were clearly observed (Fig. 1). This study provided the first proof that polyploidy did not constitute a strong apoptotic signal, since the ratio of polyploid versus euploid cells was almost the same in apoptotic and viable cells during the hours which precede the first re-replication cycle [8]. This suggests that apoptosis is triggered before mitotic segregation has taken place. This phenomenon can be explained by the fact that not only spindle microtubules but also interphase microtubules are sensitive to nocodazole. Furthermore, these results confirmed that cells exiting aberrantly from mitosis activate subsequently a p53-dependent G_1 phase [5,13], preventing further cycling of polyploid cells by blocking re-replication, not allowing another replication of the DNA without separation of the chromatids, so not allowing further polyploidization.

Once it was demonstrated that polyploid cells are not preferentially eliminated by apoptosis, we evaluated in a next step whether this would be the case for aneuploid cells induced by low concentrations of nocodazole and whether this would occur below the threshold concentrations for the induction of chromosome non-disjunction and chromosome loss that were previously demonstrated *in vitro* in PBMC by our laboratory [14,15]. For this purpose, apoptotic and viable cells were separated by magnetic microbead cell sorting combined with annexin-V staining. In the two collected populations micronuclei, chromosome loss and chromosome non-disjunction were scored in