Induction of micronuclei by a new non-peptidic mimetic farnesyltransferase inhibitor RPR-115135: role of gene mutations

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To investigate the relationship between oncogene activation and induction of micronuclei by a new non-peptidic mimetic farnesyltransferase inhibitor, RPR-115135, two isogenic cell lines, human colon cancer line HCT-116, which harbors a K-ras mutation, and spontaneously immortalized human breast epithelial cell line MCF-10A, were utilized. HCT-116 cells were transfected with an empty control pCMV vector (clone CMV-2) or with a dominant negative mutated p53 transgene (clone Mu-p53-2) to disrupt p53 function. In both clones RPR-115135 induced a significant increase in the frequency of micronucleation at concentrations that did not affect cell membrane integrity. RPR-115135 produced a significant increase in the ratio of CREST+ to CREST- micronuclei. MCF-10A cells were stably transfected with either c-Ha-ras or c-erbB-2 or both H-ras + c-erbB-2. No induction of micronuclei was observed. No induction of micronuclei was reported in human lymphocytes and in primary spinal cells obtained from 7-day chick embryos. In conclusion, RPR-115135 acts as an aneugenic agent in a complex manner, dependent upon the complement of mutations in cell regulatory genes in tumour cells and this activity may be independent of ras genotype.

Introduction

Oncogene products or proteins mediating their effects are obvious targets for cancer therapy because, by definition, these proteins are involved in malignant transformation of normal cells (Stass and Mixon, 1997; Gibbs, 2000). Of the many signal transduction mechanisms which are emerging as potential targets for drug development in cancer, prenylation of Ras family proteins, such as Ras, RhoB and Rab (all members of the Ras superfamily of proteins), is receiving particular attention from both pharmaceutical companies and academic groups (Gibbs and Oliff, 1997; Leonard, 1997; Lerner et al., 1997; Quian et al., 1997; Sebti and Hamilton, 1997; Oliff, 1999; Giraud et al., 2000; Hill et al., 2000). Interest in protein prenylation has increased because of the importance of this modification for the function of Ras proteins, GTP-binding proteins that, when mutated, contribute to the development of different types of cancers (Zachos and Spandidos, 1997). These proteins are localized at the inner surface of the cell membrane and participate in transmitting signals for growth and many other processes from the outside to the inside of the cell. Although several steps are involved in targeting Ras to the plasma membrane (activation), farnesylation by FTase is the only step that is required for Ras transforming activity (Katz and McCormick, 1997).

Single amino acid substitutions in codon 12, 13 or 61 that unmask Ras transforming potential create mutant forms of Ras which have impaired GTPase activity and are insensitive to GAP stimulation. Consequently, these oncogenic mutant Ras proteins are locked in the active, GTP-bound state, leading to constitutive, deregulated activation of Ras function. It has been estimated that 30% of all human tumours contain an activating mutation in Ras (Zachos and Spandidos, 1997). In addition, overexpression of normal Ras, which causes deregulation of wild-type Ras isoforms, is a common feature of human cancers (i.e. glioblastoma and breast cancer; Miyakis et al., 1998; Bredel and Pollak, 1999; Smith et al., 2000).

A range of farnesyltransferase (FTase) inhibitors [peptidics, pseudopeptidics, peptidomimetics or farnesyl pyrophosphate (FPP)-competitive non-peptidic inhibitors] have been synthesized or identified. The results achieved are due to the combined efforts of pharmaceutical companies and academic groups (Gibbs and Oliff, 1997; Leonard, 1997; Lerner et al., 1997; Quian et al., 1997; Sebti and Hamilton, 1997; Oliff, 1999; Giraud et al., 2000; Hill et al., 2000). The first screen for FTase inhibitors involved their evaluation in direct enzyme assays, followed by cell culture assays and subsequent testing in xenographic mice (Gibbs and Oliff, 1997; Leonard, 1997; Lerner et al., 1997; Quian et al., 1997; Sebti and Hamilton, 1997; Oliff, 1999; Giraud et al., 2000; Hill et al., 2000). Recently a number of Phase I trials have demonstrated that these compounds can be administered to cancer patients and multiple daily oral doses have been recommended (Adjei et al., 2000; Zujewski et al., 2000). Although vast resources have been allocated to this specific area of research, it is true to state that the exact mechanisms of action of FTase inhibitors remains unclear. It is possible to conclude that FTase inhibitors affect the cell in a complex manner, their effects varying not only as a function of the ras genotype of the tumour but also being dependent on other characteristics of individual tumours, such as, for example, the complement of mutations in cell cycle regulatory genes (Hill et al., 2000).

Recently, in a preliminary study (Russo et al., 1998, 1999), we have shown that a human colon cancer cell line HCT-116, when treated with a new FPP-competitive non-peptidic mimetic FTase inhibitor, namely RPR-115135, displayed severe morphological alterations (hypertrophic, vacuolated or necrotic, but not apoptotic, cells) 48–72 h after treatment. Treated cells also revealed an increase in micronucleus (MN) induction, suggesting a tendency for RPR-115135 to be an aneugenic agent (Russo et al., 1999).

The aim of this study was to evaluate the ability of RPR-115135 to increase MN induction not only as a function of the ras genotype of the tumour, but also to investigate
dependence on other characteristics of each individual tumour, such as, for example, the complement of mutations in cell cycle regulatory genes. To accomplish this we looked at the induction of MN in two different isogenic cell model systems, the human colon cancer line HCT-116, which harbours a K-ras mutation, and a spontaneously immortalized human breast epithelial cell line, MCF-10A. HCT-116 cells were transfected with an empty control pCMV vector or with a dominant negative mutated p53 transgene (248R/W) to disrupt p53 function. MCF-10A cells were stably transfected with c-Ha-ras, c-erbB-2 or H-ras + c-erbB-2. Finally, normal freshly isolated human lymphocytes stimulated with PHA/IL2 and normal primary spinal cells obtained from 7-day chick embryos were analysed.

### Materials and methods

**Chemical treatments**

RPR-115135 (C18H32NO4, mol. wt 479.58) was produced by Aventis Pharma (Centre de Recherches de Vitry, Alfortville, France). It was dissolved as a 1 mM stock solution in dimethyl sulfoxide and aliquots were stored at −20°C until needed. The compound is stable for 6 days at 37°C (Aventis Pharma, personal communication).

**Cell culture**

Human colon cancer cell line HCT-116 was grown in RPMI 1640 (Gibco BRL, Grand Island, NY) supplemented with 5% heat-inactivated fetal bovine serum (Gibco BRL) and 2 mM glutamine. Cells transfected with either empty control vector (pCMV) or vector containing a dominant negative mutant p53 transgene (248R/W) (cloned in a pCMV plasmid), to inhibit p53 function, were grown in the same medium. Generation and characterization of the HCT-116 transfectants have been described previously (Fan et al., 1997, 1998). Two clones, CMV-2 and Mu-p53-2, were examined. After γ-irradiation only parental and control transfected CMV cells but not Mu-p53 cells were able to arrest in the G1 phase of the cell cycle and accumulate p53 or p21<sub>WAF1</sub> proteins. These experiments clearly showed that the functions of p53 in the cells transfected with mutated p53 are disrupted (Table I). All systems were a kind gift of Dr P.O’Connor (Laboratory of Molecular Pharmacology, NCI–NIH, Bethesda, MD).

The MCF-10A cells were derived from a population of normal luminal mammary epithelial cells and show characteristics of normal breast epithelium. Different oncogenes (c-Ha-ras activated by a missense mutation, c-erbB-2 or c-Ha-Ras in combination with c-erbB-2) were inserted into MCF-10A cells (Ciardiello et al., 1992). p53 sequence was determined by a combination of single-strand conformation polymorphism and/or cDNA sequencing (Fan et al., 1997, 1998) and for MCF10A according to Upadhyay et al. (1995).

**G<sub>1</sub> and G<sub>2</sub> arrest were determined by flow cytometry ~16 h following exposure to 6.3 or 12.6 Gy of γ-rays (Fan et al., 1997, 1998).**

p21 (Cip1/Waf1) protein levels were determined 4 h following exposure to 6.3 or 12.6 Gy of γ-rays (Fan et al., 1997, 1998).

**Apoptosis was determined by agarose gel electrophoresis of genomic DNA and/or morphological assessment 48 h following exposure to 12.6 Gy of γ-rays (Fan et al., 1997, 1998).**

wt, wild-type; mu, mutant p53.

**G<sub>1</sub> arrest following exposure to γ-rays was transient (Fan et al., 1997, 1998).**

### Table I. Status of the p53 gene and pathway in cell lines used in the present study

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell type</th>
<th>Transfection</th>
<th>p53 gene status</th>
<th>Doubling time (h)</th>
<th>G&lt;sub&gt;1&lt;/sub&gt; arrest&lt;sup&gt;g&lt;/sup&gt;</th>
<th>G&lt;sub&gt;2&lt;/sub&gt; arrest&lt;sup&gt;g&lt;/sup&gt;</th>
<th>p21 induction&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Apoptosis&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT-116/CMV-2</td>
<td>Colon carcinoma</td>
<td>pCMV</td>
<td>wt</td>
<td>25</td>
<td>+&lt;sup&gt;h&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT-116/Mu-p53-2</td>
<td>Colon carcinoma</td>
<td>pCMV-mutated-p53</td>
<td>mut</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF-10A&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Normal luminal ductal (breast)</td>
<td></td>
<td>wt</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF-10A-H</td>
<td>c-Ha-Ras</td>
<td></td>
<td>wt</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF-10A-E</td>
<td>c-erbB-2</td>
<td></td>
<td>wt</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF-10A-H-E</td>
<td>c-Ha-Ras/c-erbB-2</td>
<td></td>
<td>wt</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary spinal cells</td>
<td>Human lymphocyte</td>
<td></td>
<td>wt</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-day chick embryos</td>
<td>Human lymphocyte</td>
<td></td>
<td>wt</td>
<td>48</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<sup>1</sup>Parenthal human colon cancer cell line HCT-116 has several oncogenic characteristics, it shows a replication error-positive phenotype [microsatellite instability related to inactivation of proteins involved in the DNA mismatch repair system (Mancuso et al., 1997)], overexpresses c-Myc (O’Connor et al., unpublished data), expresses a relatively high level of pRb (Yamamoto et al., 1999) and of cyclin E mRNA (Sutter et al., 1997), has lost normal function of the gene products of p14<sub>ARF</sub> and p16<sub>INK4a</sub> (Myohanen et al., 1998; Yang et al., 2000) and harbours a K-ras mutation at residue 13 [mut/wt (DGGE analysis) sequence GAC] (Koo et al., 1996). Finally, since HCT-116 cells have shown mutations in DNA polymerase δ mRNA, the enzyme is expected to be functionally impaired (Flohr et al., 1999). The MCF-10A cells were derived from a population of normal luminal mammary epithelial cells and show characteristics of normal breast epithelium.

<sup>2</sup>HCT-116 cells were stably transfected with empty vector (pCMV) or vector containing a dominant negative mutant p53 transgene (248R/W) (cloned into a pCMV plasmid) (Fan et al., 1997, 1998). Different oncogenes (c-Ha-ras activated by a missense mutation, c-erbB-2 or c-Ha-Ras in combination with c-erbB-2) were inserted into MCF-10A cells (Ciardiello et al., 1992).

<sup>3</sup>p53 sequence was determined by a combination of single-strand conformation polymorphism and/or cDNA sequencing (Fan et al., 1997, 1998) and for MCF10A according to Upadhyay et al. (1995).

<sup>4</sup>G<sub>1</sub> and G<sub>2</sub> arrest were determined by flow cytometry ~16 h following exposure to 6.3 or 12.6 Gy of γ-rays (Fan et al., 1997, 1998).

<sup>5</sup>p21 (Cip1/Waf1) protein levels were determined 4 h following exposure to 6.3 or 12.6 Gy of γ-rays (Fan et al., 1997, 1998).

<sup>6</sup>Apgoptosis was determined by agarose gel electrophoresis of genomic DNA and/or morphological assessment 48 h following exposure to 12.6 Gy of γ-rays (Fan et al., 1997, 1998).

<sup>7</sup>wt, wild-type; mu, mutant p53.

<sup>8</sup>G<sub>1</sub> arrest following exposure to γ-rays was transient (Fan et al., 1997, 1998).
were washed twice with phosphate-buffered saline (PBS). Cells were fixed with 4% paraformaldehyde in PBS for 30 min at room temperature and then stained. DAPI staining was performed according to Linn et al. (1977). Briefly, cell culture slides were incubated in a 1:200 solution of 1 µg/ml DAPI in methanol and observed on a Leica microscope (magnification ×400), equipped with a UV detector at a wavelength of λ360 nm. DAPI identifies the A-T couples of DNA as blue-white fluorescence. One thousand cells were scored per slide stained with DAPI to evaluate MN induction. Student’s t-test was used to assess statistical significance (not significant, P > 0.05).

The highest tested concentration of RPR-115135 was the maximum tolerated by the cells. Increases, even small, in concentration produced unwanted effects, such as extensive nuclear fragmentation, cell division inhibition and detachment of cells from the slides (Giemsia stained standard preparation).

In a second set of experiments CREST antiserum for kinetochore staining and propidium iodide for nuclei/micronucleus counterstaining were used as previously reported (Nüssse et al., 1989). Generally accepted criteria for identifying MN (Bonatti et al., 1986) were adopted to rule out artefacts. The specificity of propidium iodide staining further guaranteed that the structures scored as MN contained DNA. A minimum of 100 MN were examined for the presence of kinetochore-positive signals.

### Results

#### Cell cytotoxicity

Since we have previously reported IC50 values obtained in the HCT-116 cell line system (Russo et al., 1999), we began by investigating the sensitivity to RPR-115135, over a 6 day exposure, of MCF-10A parental and of MCF-10A-H, MCF-10A-E and MCF-10A-HE cells. Interestingly, all cells appeared sensitive to RPR-115135, however, the most sensitive was MCF-10A-H and the most resistant MCF-10A (~10 times difference) in terms of IC50 values (Table II). Although the untransfected cells (MCF-10A) were 10 times less sensitive to RPR-115135 than MCF-10A-H cells, they displayed a moderate sensitivity to RPR-115135 (Table II; IC50 = 8.0 µM).

The IC50 value for MCF-10A-H was in the same range of concentrations obtained in the HCT-116 isogenic cell system (0.78 versus 0.55 µM).

When MCF-10A-H cells were treated with 10 µM RPR-115135 in the MTS assay the percentage of cells surviving was ~8%. In a clonogenic assay no colonies were detected when treated at this concentration (data not shown). Evaluation by Giemsa staining of cells treated with 10 µM RPR-115135 for 48 h showed 100% of cells with a severely altered morphology. From these data a concentration of RPR-115135 of 1.0 µM was chosen to investigate the time course, cell cycle responses and induction of MN in MCF-10A cells. The dose dependence of MN induction was evaluated in HCT-116 cells and in human lymphocytes.

### Time course

We have previously demonstrated in time course experiments conducted over a 6 day exposure to 10 µM RPR-115135 that clones HCT-116-CMV-2 and HCT-116-Mu-p53-2 were able to grow for up to 72 h following administration of the drug (Russo et al., 1999), but thereafter, approaching saturation density, a clear growth inhibition was observed.

The same experiments performed in the MCF-10A cell system showed again that the most sensitive clone was MCF-10A-H, while the other cells were moderately sensitive or resistant. The kinetics of the time–growth curve were similar to those obtained in HCT-116 cells (Figure 1A–D; Russo et al., 1999).

Growth inhibition could not easily be accounted for on the basis of a specific cell cycle arrest phenotype, as assayed by flow cytometry in the two cell systems (Russo et al., 1999; and data not shown for MCF-10A cells). These observations support the hypothesis that RPR-115135 did not work by inhibiting cell growth at any specific phase of the cell cycle. In addition, no induction of apoptosis was observed (TEM analysis, DAPI and Giemsia staining and flow cytometry; data not shown)

**Induction of micronuclei**

Since previous observations (Russo et al., 1998, 1999) supported the hypothesis that RPR-115135 did not work by inhibiting cell growth of proliferating cells at any specific phase of the cell cycle and that the growth inhibition effect of RPR-115135 could not be easily ascribed to significant induction of apoptosis, we looked at other possible alterations involved in cell cytotoxicity, such as induction of MN.

The frequencies of MN obtained by scoring DAPI stained slides after treatment with different concentrations of RPR-115135 (0.1–10 µM) for 48 h in clones CMV-2 and Mu-p53-2 are reported in Figure 2B. Figure 2A reports cell survival. Only the highest concentrations (10 µM) caused a statistically significant cytotoxicity in both clones. The same cell preparations were used to evaluate the percentage of surviving cells and the frequency of MN. Regarding induction of MN, univariate analysis of variance [multiple comparisons (Dunnett’s t-test treating one group as a control and comparing all other groups with it)] revealed that the only statistically significant result was obtained on treating cells with 10 µM RPR-115135 (P = 0.001 for clone CMV-2; P = 0.013 for clone Mu-p53-2).

In the light of these data, HCT-116 cells were treated with 10 µM RPR-115135 in a time dependence experiment. A significant increase in induction of MN to a similar extent was observed by scoring propidium iodide stained slides of both clone CMV-2 and Mu-p53-2. The increase started 24 h after treatment, when no cytotoxic effect was seen, with the maximum number of MN being reached 2 days after treatment; it remained at a plateau level for an additional 2 days of treatment (Figure 3), after which, looking at the growth kinetics, a clear growth inhibition induced by RPR-115135 was evident. After 5 or 6 days treatment the MN count became technically difficult because of a tendency of the treated cells to form small compact clusters with highly condensed chromatin (data not shown).

The evaluation of cell membrane integrity (10 µM RPR-115135 over 6 days) revealed that the percentage of HCT-116 cells able to exclude trypan blue dye was ~85% (cellular debris not considered), suggesting that those cells with MN had substantially intact cell membranes. These data were supported by looking at cell morphology on Giemsia stained slides (data not shown).

### Table II. Cell cytotoxicity induced by RPR-115135 in MCF-10A isogenic human breast epithelial cells

<table>
<thead>
<tr>
<th>Cell clone</th>
<th>IC50 (µM)</th>
<th>Sensitivitya</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-10A</td>
<td>8.0 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>MCF-10A-H</td>
<td>0.8 ± 0.1</td>
<td>10.0</td>
</tr>
<tr>
<td>MCF-10A-E</td>
<td>5.7 ± 0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>MCF-10A-H-E</td>
<td>2.8 ± 0.5</td>
<td>2.9</td>
</tr>
</tbody>
</table>

IC50 values are reported as means ± SE of at least three independent experiments, each performed in sextuplicate. The MTS assay was performed after 6 days continuous exposure.

aRatio = (IC50 parental)/IC50 transfctant.

bStatistically significant (P < 0.001), according to Student’s t-test, versus parental.

Micronucleus induction by a FTase inhibitor
Since MN can be formed by chromosomal breakage or chromosome loss (Nüsse et al., 1989), an antibody obtained from serum of patients with the autoimmune disease CREST syndrome was used (Nüsse et al., 1989). Centromeres in MN were immunologically visualized with anti-kinetochore antibodies from CREST patients. The CREST serum contains antibodies to a specific protein of the kinetochore region of chromosomes. Cells with CREST-containing MN are principally induced by aneuploidy-inducing agents (e.g. vincristine), while MN lacking CREST labeling are induced by clastogenic agents (e.g. mitomycin) (Figure 4). Figure 5 shows the frequency (per 1000 cells) of total MN and CREST+/−MN induced by 10 μM RPR-115135 at different times of treatment in both the CMV-2 and Mu-p53-2 clones. The comparison between total MN and kinetochore-positive MN (CREST+) frequencies showed that RPR-115135 induced predominantly CREST+ MN in both the CMV-2 and Mu-p53-2 clones (Figure 5). With respect to a culture time effect, no statistically significant differences (P > 0.05, Student’s t-test) in the percentage of CREST+ MN were observed in relation to incubation time (1–4 days) with RPR-115135 when all data were analysed.

These data show a tendency for RPR-115135 to be an aneugenic agent.

The frequencies of MN in the MCF-10A cell system were scored after 48 h, when no significant cell death was present after treatment with 1 μM RPR-115135. No induction of MN was observed in MCF-10A cells (Figure 6).

The evaluation of cell membrane integrity (1 μM RPR-115135 over 6 days) revealed that the percentage of MCF-10A cells able to exclude trypan blue dye was ~79.3%, of MCF-10A-H cells 52.5%, of MCF-10A-E cells 100% and of
Micronucleus induction by a FTase inhibitor

Fig. 2. (A) Dose-dependent sensitivity to different concentrations of RPR-115135 in clones CMV-2 and Mu-p53-2 after 48 h treatment. (B) Dose-dependent induction of MN after treatment with different concentrations of RPR-115135 in clones CMV-2 and Mu-p53-2. Univariate analysis of variance, according to Dunnett’s t-test, treats one group as a control and compares all other groups with it: for 0.1 µM RPR-115135 $P = 0.989$; for 1.0 µM $P = 0.761$; for 10.0 µM $P = 0.001$.

Fig. 3. Frequency of MN (per 1000 cells) in clones (A) CMV-2 and (B) Mu-p53-2 after treatment with 10 µM RPR-115135 for different periods. MN were stained with propidium iodide. 1000 cells were scored for each slide, each experiment was performed at least in duplicate and two different independent experiments were performed. Means ± SE are given. Statistically significant ($P < 0.001$), according to Student’s t-test, versus their own corresponding control.

Fig. 4. Frequency (per 1000 cells) of kinetochore-positive MN and kinetochore-negative MN over total MN in clone CMV-2 after treatment with vincristine (0.05 µM for 1 h) or mitomycin C (0.5 µM for 17 h). A sample of 100 MN was scored for each slide, each experiment was performed in duplicate and two different independent experiments were performed.

Fig. 5. Frequency (per 1000 cells) of kinetochore-positive MN and kinetochore-negative MN over total MN in clones (A) CMV-2 and (B) Mu-p53-2 after treatment with 10 µM RPR-115135 for different periods. A sample of 100 MN was scored for each slide, each experiment was performed in duplicate and two different independent experiments were performed.

MCF-10A-EH cells 89% (cellular debris not considered). Only in H-ras transfected cells ~50% did not have intact cell membranes, suggesting massive necrosis and perhaps explaining the absence of MN induction. These data were confirmed by analysing the cell morphology of Giemsa stained cells. About 40% of MCF-10A-H cells showed a typical picture of necrosis.

In human lymphocytes the IC$_{50}$ concentration of RPR-115135 was 10 µM, while 0.1 and 1.0 µM had no effect on cell proliferation (48 h treatment).

Table III reports the number of MN scored; a univariate analysis of variance [multiple comparisons (Dunnett’s t-test treating one group as a control and comparing all other groups with it)] revealed that no data were statistically significant ($P > 0.05$).

Induction of MN was also studied in primary normal chick spinal cells obtained from 7-day chick embryos. Treatment with 10 µM RPR-115135 over 6 days induced massive cell death, while 1.0 µM was well tolerated over 6 days treatment. After 48 h treatment with 1 µM RPR-115135 cell survival was ~75%. MN were also scored after 48 h treatment with 1.0 µM RPR-115135 (Figure 7). No induction of MN was observed.
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Fig. 6. Frequency of MN (per 1000 cells) in the isogenic MCF-10A cell system after treatment with 1 µM RPR-115135 for 48 h. A sample of 1000 cells was scored for each slide, each experiment was performed at least in duplicate and two different experiments were performed. Means ± SE are given. Not statistically significant (P > 0.05), according to Student’s t-test, versus their own corresponding control.

Table III. Induction of MN (per 1000 cells) in human lymphocytes treated for 48 h with different concentrations of RPR-115135

<table>
<thead>
<tr>
<th>RPR-115135 (µM)</th>
<th>No. MN donor 1 (mean ± SE)</th>
<th>No. MN donor 2 (mean ± SE)</th>
<th>Total no. MN (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>8.5 ± 0.5</td>
<td>16.5 ± 1.5</td>
<td>12.5 ± 2.4</td>
</tr>
<tr>
<td>0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.0 ± 4.0</td>
<td>18.5 ± 2.5</td>
<td>20.3 ± 2.2</td>
</tr>
<tr>
<td>1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.0 ± 4.0</td>
<td>13.0 ± 2.0</td>
<td>14.5 ± 2.0</td>
</tr>
<tr>
<td>10.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.5 ± 1.5</td>
<td>14.5 ± 0.5</td>
<td>20.0 ± 3.2</td>
</tr>
</tbody>
</table>

Univariate analysis of variance, according to Dunnett’s t-test, treats one group as a control and compares all other groups with it.

<sup>a</sup>P = 0.124.
<sup>b</sup>P = 0.897.
<sup>c</sup>P = 0.139.

Discussion

The role of RPR-115135, a FPP-competitive FTase inhibitor, in the induction of MN was analysed in cells having different origins (human or chick) and/or carrying different mutations. The aim of the study was not only to evaluate the ability of RPR-115135 to increase MN induction as a function of the ras genotype of the tumour, but also to investigate dependence on other characteristics of each individual tumour, such as, for example, the complement of mutations in cell cycle regulatory genes.

To accomplish this, two well-characterized isogenic cell lines were used. The first model was the HCT-116 human isogenic colon cancer cell line with intact versus disrupted p53 function. In this system RPR-115135 is able to induce a significant number of CREST+/MN, suggesting a potential ability of RPR-115135 to act as an aneugenic agent. This ability is independent of p53. In this system evaluation of cell membrane integrity (10 µM RPR-115135 over 6 days) revealed that the percentage of cells able to exclude trypan blue dye was ~85%, suggesting that MN induction was in cells with substantially intact cell membranes. Giemsa staining supported this observation.

However, the parental human colon cancer cell line HCT-116 has several other oncogenic characteristics (Table I), including loss of ARF normal function. ARF is involved in tumour surveillance (ARF antagonizes Mdm2 to activate p53; Sherr, 2000) and its expression is activated by abnormal mitogenic signals induced by overexpression of oncoproteins such as Myc and Ras (Lloyd, 2000; Sherr, 2000). Loss of the ARF checkpoint subverts this form of cell-autonomous tumour surveillance and allows proteins such as Ras and Myc to function as ‘pure’ proliferation enhancers. Recently,
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