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Research article

The farnesyl transferase inhibitor RPR-130401 does not alter radiation susceptibility in human tumor cells with a K-Ras mutation in spite of large changes in ploidy and lamin B distribution

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Abstract

Background: Growth inhibition by RPR-130401, a non-peptidomimetic farnesyltransferase inhibitor, was investigated without or with combined exposure to ionizing radiation in three human tumor cell lines (HCT-116, MiAPaCa-2 and A-549) bearing a point mutation in the *K-Ras* gene.

Results: RPR-130401 inhibited cell growth with an IC_{50} of 50 nM (HCT-116), 120 nM (MiAPaCa-2) and 710 nM (A-549), with a poor incidence of apoptosis. The drug brought about G1 and S phase depletion together with arrest of cells in G2 phase and induced a significant accumulation of hyperploid cells showing active S phase DNA synthesis, with HCT-116 and A-549 cells being the most and least responsive, respectively. The drug also produced dramatic changes of the nuclear lamin B pattern, without lamin B cleavage and perturbation of the actin cytoskeleton. On the other hand, RPR-130401 elicited strictly additive interaction in combined treatment with ionizing radiation with regard to cell kill, altered cell cycle progression and induced hyperploidy.

Conclusions: The data suggest that disruption of orderly progression through mitosis and cytokinesis, is a major outcome of drug action and that this effect proceeds from inhibition of lamin B farnesylation. It is anticipated from the strict additivity of RPR-130401 and radiation that neither induced radiation resistance nor acute or late complications of radiotherapy, should occur in combined treatment with RPR-130401.

Background

For over ten years chemo-radiotherapeutic combinations have evolved as prevalent modalities in the cure of solid tumors. The rationale for these treatments relies mostly on the drugs' ability to sterilize disseminated metastases (spatial cooperation) and on radiation-drug interaction for improved local control of the primary tumor. Supraadditive interaction, often resulting from impaired repair of radiation-induced sublethal damage, may lead to limiting toxicities. In contrast, pure additivity of the treatments may conceivably be turned to advantage because the dose-dependent response of cells to radiation includes a quadratic term. This is of concern, for example, to farnesyl transferase inhibitors (FTIs) of which six have already been tested in phase I clinical trials [1,2] with most attention being paid to the non-peptidomimetic R115777 [3–

5]. As one may expect a cytostatic rather than a cytotoxic effect from FTIs, assays of combined modality treatment with cytotoxic agents applied in close temporal proximity should be performed. This prompted us to investigate the cells' response to RPR-130401 (Scheme 1), a non-peptidomimetic FTI issued from chemical optimization from series selected by random screening at Rhône-Poulenc-Rorer Co. [6], both alone or in concomitant association with ionizing radiation.

A comprehensive approach to these problems requires an insight into the molecular targets of drug action. Together with mutation or deletion of the *p53* tumor suppressor gene, mutations in one of the four *Ras* genes (*H-*, *N-*, K_A- and K_B-*Ras*) represent the most frequent genetic alterations in human cancers [7] with predominance in pancreatic (90%), colorectal (50%) and lung (20%) tumors (for a review on ras mutations in relation to oncogenic transformation see [2]). Activation of Ras proteins requires prenylation of the CAAX sequence at their C-terminus, to ultimately permit their association with other proteins [8] or with the inner face of the plasma membrane [9]. A key step in this process is catalyzed by farnesyltransferase (FTase). The K-Ras protein can alternatively be prenylated by type I geranylgeranyltransferase (GGTase I), a closely related enzyme [10].

FTase has been a very attractive target for antitumor drug discovery because prenylation is required for oncogenic Ras to transform cells [11–13]. Numerous inhibitors have been developed, including FPP analogs, CAAX peptide analogs, bisubstrate analogs [14,15] and more recently GGTase I inhibitors [16]. However, it is now known that a range of non-Ras proteins are targets for prenylation [17–19]. In spite of this, the quest for specific FTase-targeting drugs that would not affect GGTase is still pertinent, because geranylgeranylation of normal cellular proteins is five to ten times more prevalent than farnesylation [20,21].

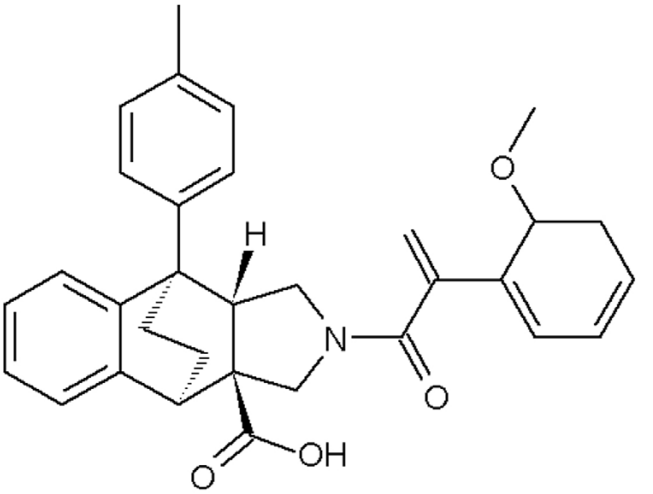
RPR-130401 acts as a competitive inhibitor of the FPP substrate with respect to the farnesylated proteins, with an IC_{50} of 28 nM for the inhibition of K-Ras prenylation *in vitro*[6], and has demonstrated *in vivo* antitumor activity in mice bearing human carcinoma xenografts [22]. RPR-130401 is highly selective (more than 300-fold) for FTase with regards to GGTase, an unexpected result since GGTase can accommodate and transfer both FPP and geranylgeranyl pyrophosphate to CAAX-motifs in proteins [23]. Consistently, RPR-130401 efficiently inhibits Ras farnesylation in cells but does not block geranylgeranyl transfer to Ras [24]. RPR-130401 is also very efficient in inhibiting lamin B farnesylation [6]. The lack of protein substrate specificity is of particular interest for the development of this series of FTIs, as non-Ras farnesylated proteins may participate in transformation by Ras-dependent or -independent pathways. The discovery of prenylation-dependent oncogenic protein tyrosine phosphatases harboring a CAAX-box [25] strengthens the interest for such FTIs as RPR-130401.

For studies with RPR-130401 we chose three cell lines bearing a *K-Ras* allele with a point mutation, namely, the human colon adenocarcinoma HCT-116 with mutation at codon 13 [26], the human pancreatic carcinoma MiAPaCa-2 with mutation at codon 12 [27], and the human lung carcinoma A-549 with another mutation at codon 12 [28]. Cell growth or survival, cell cycle progression, ploidy, lamin B structure in nuclear lamina, cytoskeleton framework, and lamin B fragmentation were taken as an endpoint in single or combined treatment with ionizing radiation. The data show that RPR-130401 affects the integrity of the lamin B network, resulting in an aberrant onset of mitosis and cytokinesis and ensuing hyperploidy without significant changes in radiation susceptibility.

Results

Growth inhibition by RPR-130401

The response of HCT-116, A-549 and MiAPaCa-2 cells to RPR-130401 was investigated through growth inhibition assays with exponentially growing subcultures. Cells were exposed to the drug for up to 7-days at concentrations in the range 2.5–7,000 nM. RPR-130401 was found to inhibit cell growth with an IC_{50} of 50 nM for HCT-116, 120 nM for MiAPaCa-2 and 710 nM for A-549 cells. The effect correlated with a pronounced lengthening of the cells' doubling time (Figure 2).



Scheme 1
Chemical Structure of RPR-130401

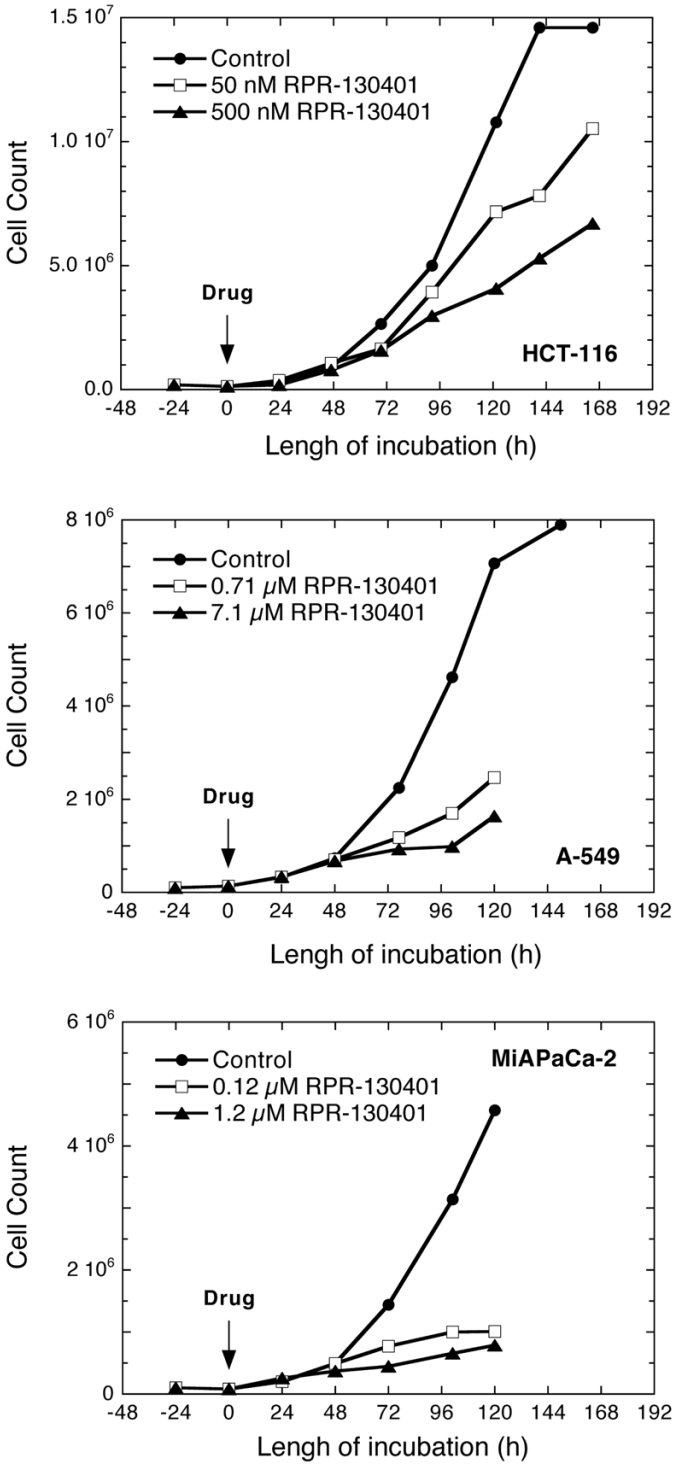


Figure 2
Growth inhibition by RPR-130401 in asynchronously growing HCT-116, A-549 and MiAPaCa-2 cells. Mid-log phase cells were seeded (2×10^5 cells in 25 cm^2 flasks) and incubated for 24-h prior to introduction of RPR-130401. The drug was present for up to the time of trypsinization and cell scoring. RPR-130401 induced a pronounced lengthening of the cell doubling time, namely, for HCT-116 cells, 23-h (50 nM drug = $1 \times IC_{50}$) or 30-h (500 nM drug) *vs.* 18-h in drug-free controls; for A-549 cells, 30-h (0.71 μM drug = $1 \times IC_{50}$) or 35-h (7.1 μM drug) *vs.* 22-h in drug-free controls; for MiAPaCa- 2 cells, 35-h (0.12 μM drug = $1 \times IC_{50}$) or 40-h (1.2 μM drug) *vs*. 23-h in drug-free controls.

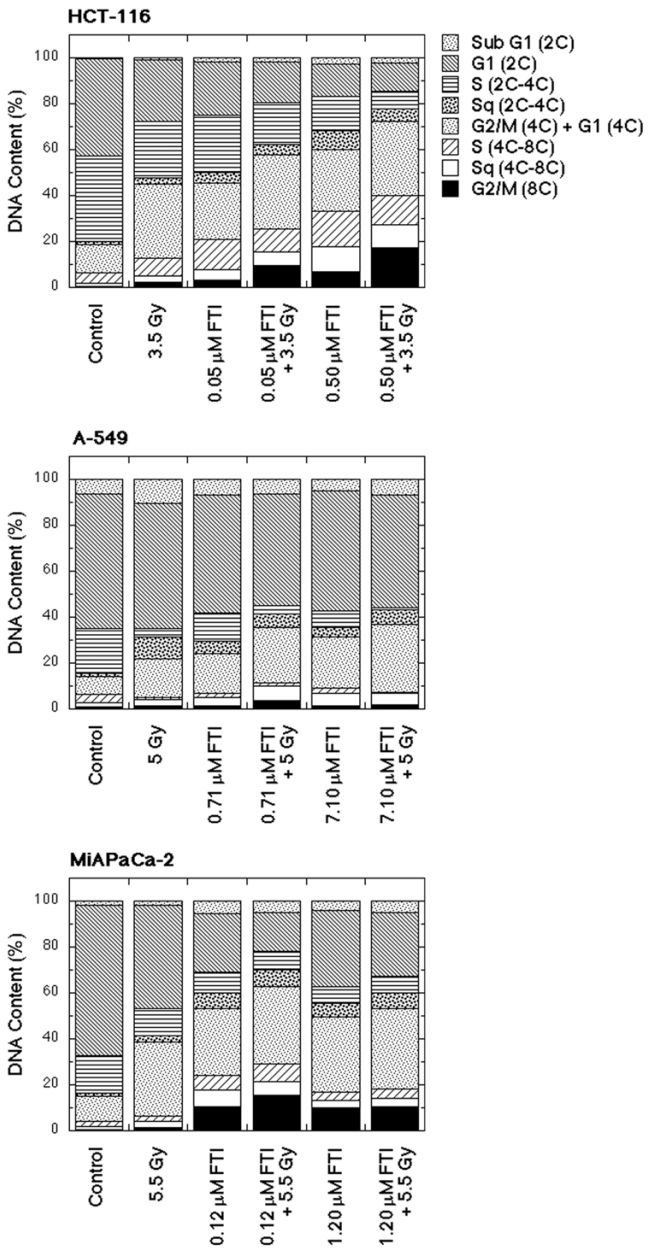


Figure 3
Effect of RPR-130401 on cell cycle progression. Cells were grown for a total of 5-days without or with $1 \times IC_{50}$ or $10 \times IC_{50}$ RPR-130401. At day 4, part of samples was irradiated to the dose indicated and allowed to rest in the incubator for an additional 24-h prior to harvest. The DNA content was determined from flow cytometric analysis of propidium iodide staining and BrdUrd incorporation. 10^4 nuclei were analysed for each sample. 2C, 4C and 8C, diploid, tetraploid and octoploid cells. Sq, quiescent (arrested in synthesis) S-phase cells.

Altered cell cycle progression by RPR-130401 and radiation

To investigate cell cycle redistribution by RPR-130401, HCT-116, MiAPaCa-2 and A-549 cells were exposed for 5-days to $1 \times IC_{50}$ or $10 \times IC_{50}$ FTI (relative to the cell line of interest) and submitted or not to γ-rays 24-h before harvest. BrdUrd was incorporated at the end of treatment for S-phase cell labeling. The three cell lines were not equally responsive to drug (Figure 3). RPR-130401 induced accumulation of HCT-116 cells in G2-M phase, with a concentration-dependent increase in the ploidy number and a decline in the normodiploid G1- and S-phase content. These observations correlate those made by other authors using Ras-mutated human pancreatic cells and L-744,832 [29] or human lung cancer cells and FTI-2153 [30]. MiAPaCa-2 cells showed a similar response at low ($1 \times IC_{50}$) drug concentration. However, with this cell line both the G1 phase depletion and the increase in the ploidy number, were substantially less pronounced at high ($10 \times IC_{50}$) than at low ($1 \times IC_{50}$) drug concentration. Finally, with the exception of the pre-mitotic arrest, the effect of RPR-130401 on cell cycle progression and ploidy change was comparatively minor in A-549 cells. Irradiation promoted drug-induced effects. Incidentally, flow cytometric analysis did not provide evidence of a significant amount of the sub-G1 DNA fragments expected from apoptosis.

Radiation survival in the presence of RPR-130401

Two methods were used to determine whether RPR-130401 resulted or not in an altered radiation survival. For lengths of drug exposure in excess of 12-h (1- to 5-days), cells were trypsinized and plated following treatment to preclude artifacts due to cell multiplicity. Short contact with drug (1-, 2-, 3- or 12-h) was performed in the most sensitive cell line only, i.e., in HCT-116 cells. In that case, cells were plated prior to treatment and the data corrected for changes in cell multiplicity (see Materials and Methods) where necessary. A total of 78 survival curves were generated and fitted to eq. (2) (see Materials and Methods) for data analysis. Irrespective of the method used, exposure of cells to RPR-130401 did not produce any significant modification of radiation sensitivity relative to controls, even with the largest drug concentration and the longest length of drug exposure (Figure 4).

Effects of RPR-130401 on lamin B distribution

Altered distribution of lamin B following exposure to the FTI was investigated in HCT-116, A-549 and MiaPaCa-2 cells. Cells were exposed for 5-days to $1 \times IC_{50}$ or $10 \times IC_{50}$ RPR-103401, submitted or not to γ-rays 24-h before harvest and lamin B was subsequently probed by immunofluorescence. Typical results are shown in Figure 5.

Control cells (Figure 5A,5D & 5G) showed a nuclear distribution of lamin B with localization to the nuclear lam-

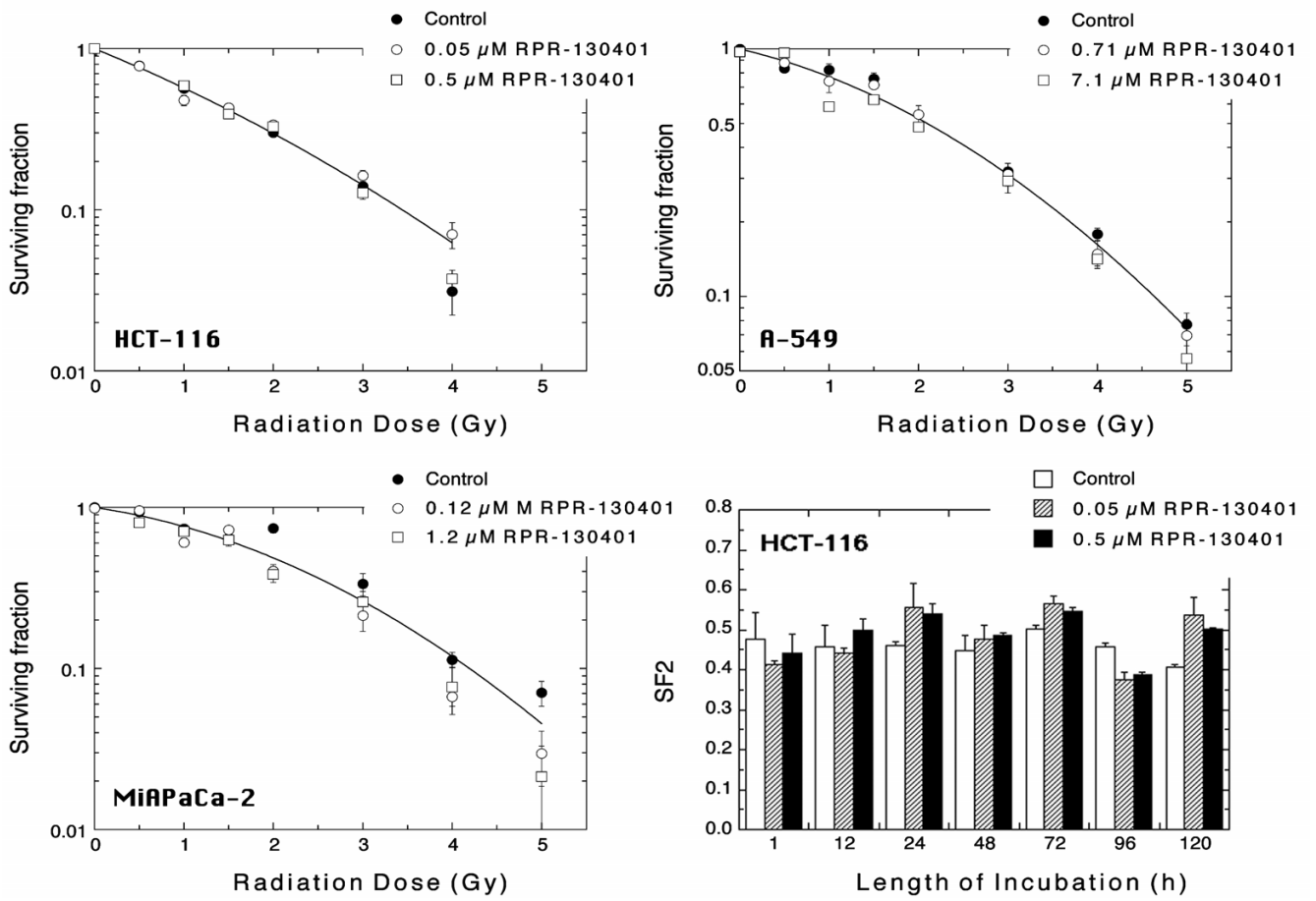


Figure 4
Typical radiation survival curves for asynchronous growing cells without (•) or with exposure to $1 \times IC_{50}$ (○) or $10 \times IC_{50}$ (□) RPR-130401. Cells were plated, exposed to the drug for 3-days and irradiated. Each flask was trypsinized immediately after irradiation and 800 to 1,000 cells were replated in drug-free medium for colony formation assays. To account for the loss of plating efficiency du to drug exposure, survival values were normalized relative to the surviving fraction for drug alone. The curves were drawn for best fit to a linear quadratic equation (eq. (2), see Materials and Method) taking all data points into consideration. Found: $\alpha = 0.518 \pm 0.036$ Gy^{-1}, $\beta = 0.044 \pm 0.016$ Gy^{-2} for HCT-116 cells; $\alpha = 0.193 \pm 0.036$ Gy^{-1}, $\beta = 0.065 \pm 0.014$ Gy^{-2} for A-549 cells; $\alpha = 0.189 \pm 0.058$ Gy^{-1}, $\beta = 0.086 \pm 0.026$ Gy^{-2} for MiAPaCa-2 cells. Each data point came from an average over three flasks or more. *Bars, SD*. The figure at bottom right shows the surviving fraction at 2 Gy (SF_2) of HCT-116 cells in the presence of RPR-130401. The conditions were the same as above. Data were corrected for the expression of potentially lethal damage [82].

ina as indicated by a ring-like staining at the nuclear periphery. During mitosis the lamin was either cytoplasmic (Figure 5D) or poorly detectable (Figure 5G,5H). The pattern of lamin B distribution was markedly altered following incubation with RPR-130401, depending on the cell line. For HCT-116 (Figure 5B,5C) and A-549 cells (Figure 5E,5F), the lamin B staining was still nuclear with the characteristic ring-like structure but, in addition to giant nuclei typical of treated HCT-116 cells (Figure 5C), cell doublets with no proper separation of the daugther nuclei after telophase were observed (Figure 5F). MiAPaCa-2 cells showed a different pattern in response to RPR-130401 (Figure 5H,5H'). With these cells, lamin B was not strictly confined in the nucleus as indicated by cytoplasmic patches (Figure 5H). Examination of DAPI-stained nuclei confirmed the absence of DNA in these patches (Figure 5H'). Lamin B figures were not appreciably modified by exposure to ionizing radiation; however, radiation induced the formation of micronuclei in relation to mitotic cell death (data not shown).

Effects of RPR-130401 on actin and tubulin networks in HCT-116 cells

Some FTIs reportedly affect the actin cytoskeleton *via* the Rho (a Ras-related GTP-binding protein) pathway [31] and Rho-B, an endosomal Rho protein involved in recep-

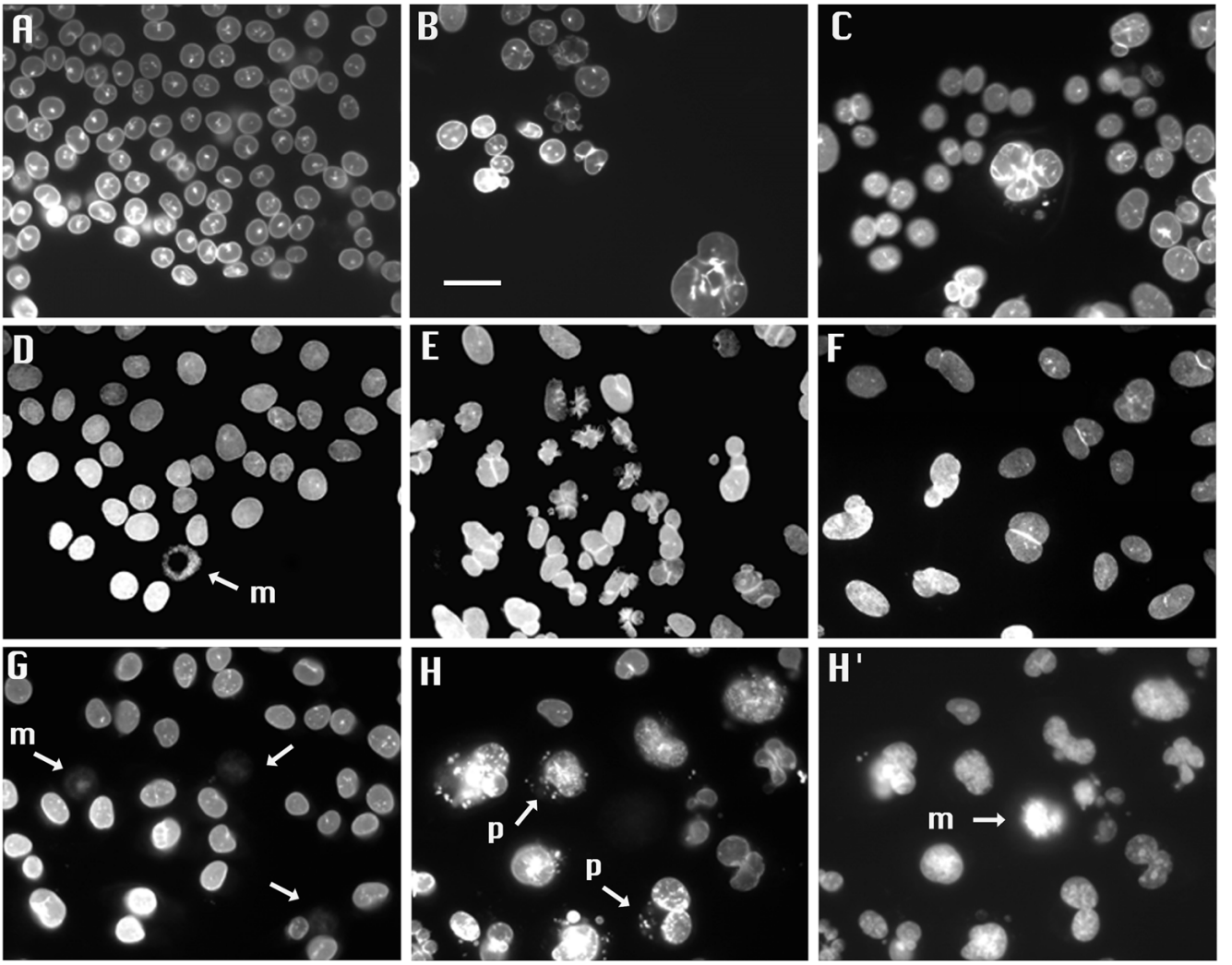


Figure 5
Immunofluorescent detection of lamin B in control (A, D, G) and treated (B, C, E, F, H, H') HCT-116 (B, C), A-549 (E, F) and MiAPaCa-2 cells (H, H'). Cells were grown on coverslips for 5-days without or with $1 \times IC_{50}$ (B, E, H, H') or $10 \times IC_{50}$ (C, F) RPR-130401. Cells were then fixed and incubated with an anti-lamin B monoclonal antibody, then with an Alexa®-conjugated secondary antibody. To visualize nuclei, the cells were counter-stained with DAPI (H'). Cells were viewed on a Zeiss microscope and photographed with Ilford HP-5 film. m, mitosis; p, cytoplasmic patches. The bar (B) represents 20 μm.

tor trafficking, has recently been suggested as an important mediator of the antineoplastic potential of FTIs [19,32]. We therefore sought to investigate the effects of 50 nM or 500 nM RPR-130401 on the distribution of actin and tubulin networks in HCT-116-cells exposed to RPR-130401 and/or radiation. Pictures obtained using rhodamine-phalloidin and immunofluorescent labeling of isothiocyanate-conjugated tubulin in control and treated cells, are shown in Figure 6.

L-739,749, another FTI, was earlier shown to induce rapid morphological reversion of rat fibroblasts (Rat1-ras) transformed with H-ras [33]. Such phenotypic reversion was not observed using RPR-130401 in HCT-116 cells. Normal membrane ruffles, circumferential actin bundles and focal adhesions as seen in control HCT-116 cells (Figure 6A), were still present in cells incubated for as long as 5-days in the presence of RPR-130401 (Figure 6B). The size of the nuclei and the number of giant cells grew in parallel in the presence of the drug, up to threefold relative to controls. The stress fibers and the tubulin network were normal in these cells (Figure 6B & 6D). Examination of bisbenzimide-stained nuclei showed the absence of apoptotic bodies (Figure 6A′,6B′), and no vesicles were formed in the cytoplasm of treated cells.

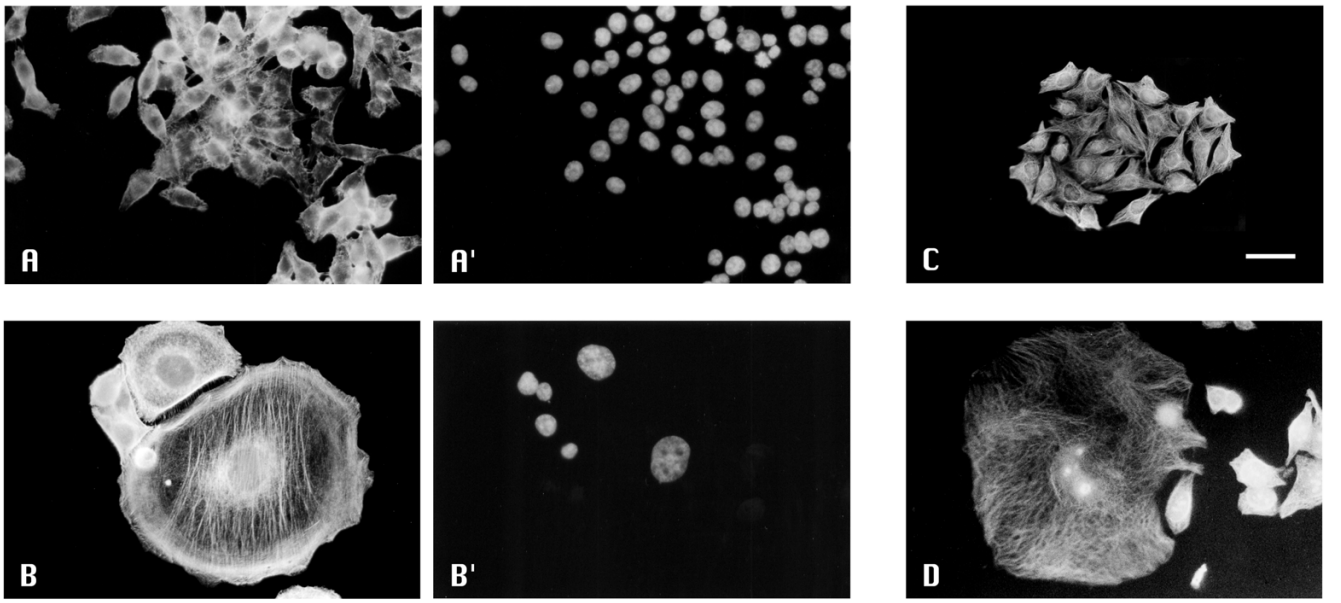


Figure 6
Immunofluorescent visualization of cytoskeleton components in control (A, A', C) and treated (B, B', D) HCT-116 cells. Cells were grown on coverslips for 5-days without or with 500 nM RPR-130401. The cells were subsequently given 3.5 Gy γ-rays and allowed to rest in the incubator for 24-h. Cells were then fixed and incubated with TRITC-labeled phalloidin (A, B), or with an anti-α tubulin FTIC-conjugated antibody (C, D). Nuclei were counter-stained with bisbenzimide (A', B'). Cells were viewed as in Figure 5. The bar (C) represents 20 μm.

RPR-130401-induced modification of the HCT-116 karyotype

Activation of Ha-ras gene expression in mouse NIH3T3 cells is known to result in genomic instability, identified from chromosome aberrations including acentric fragments, multicentric and double-minute chromosomes [34,35]. We therefore investigated the effects of 50 nM or 500 nM RPR-130401 on the ploidy and chromosome integrity in HCT-116-cells exposed to RPR-130401 and/or radiation. Figure 7 shows the distribution of the chromosome score within individual metaphases of colcemid-treated cells after 1-day of contact with RPR-130401. Control cells showed a near-diploid karyotype with a modal number of chromosomes of 45. Excess ploidy increased with the drug concentration, the radiation dose and the length of drug exposure. The mitotic index decreased during treatment (data not shown), thus confirming that the accumulation of HCT-116 cells was in the G2 phase.

However, no chromosome aberration was seen at this stage using FISH analysis with all human centromeres probes. All chromosomes in the metaphases examined displayed one unique centromere, even in metaphases containing up to 200 chromosomes (data not shown).

Western blot analysis of lamin B in treated cells

Flow cytometry (Figure 3) and DAPI fluorescence of nuclei (Figure 5), did not provide evidence of a significant level of apoptosis in cells exposed to RPR-130401. In some instances, however, the pictures obtained from immunofluorescence analysis of the lamin B status in cells exposed for 5-days to RPR-130401, suggested breakdown of nuclear lamina, and it has been shown recently that inhibition of protein prenylation may cause apoptotic cell death in pancreatic cells [36]. As lamin B cleavage into 46 kDa fragments is a hallmark of apoptosis [37], we performed a Western blot determination of lamin B in extracts from HCT-116, A-549 and MiAPaCa-2 cells exposed for 5-days to $10 \times IC_{50}$ RPR-130401. The results (Figure 8) unambiguously show complete absence of lamin B cleavage.

Discussion

The main effects of RPR-130401 against the three K-Ras mutated cell lines used in this study were *(i)* a pronounced cytostatic effect, with an IC_{50} in the range 50–710 nM; *(ii)* accumulation of cells in G2 phase, with G1 and S phase depletion; *(iii)* induction of a large amount of hyperploid cells (HCT-116 and MiAPaCa-2) showing active S-phase DNA synthesis, together with some giant cells; *(iv)* profound alteration of the lamin B pattern, without cleavage of the protein; *(v)* no alteration of the micro-

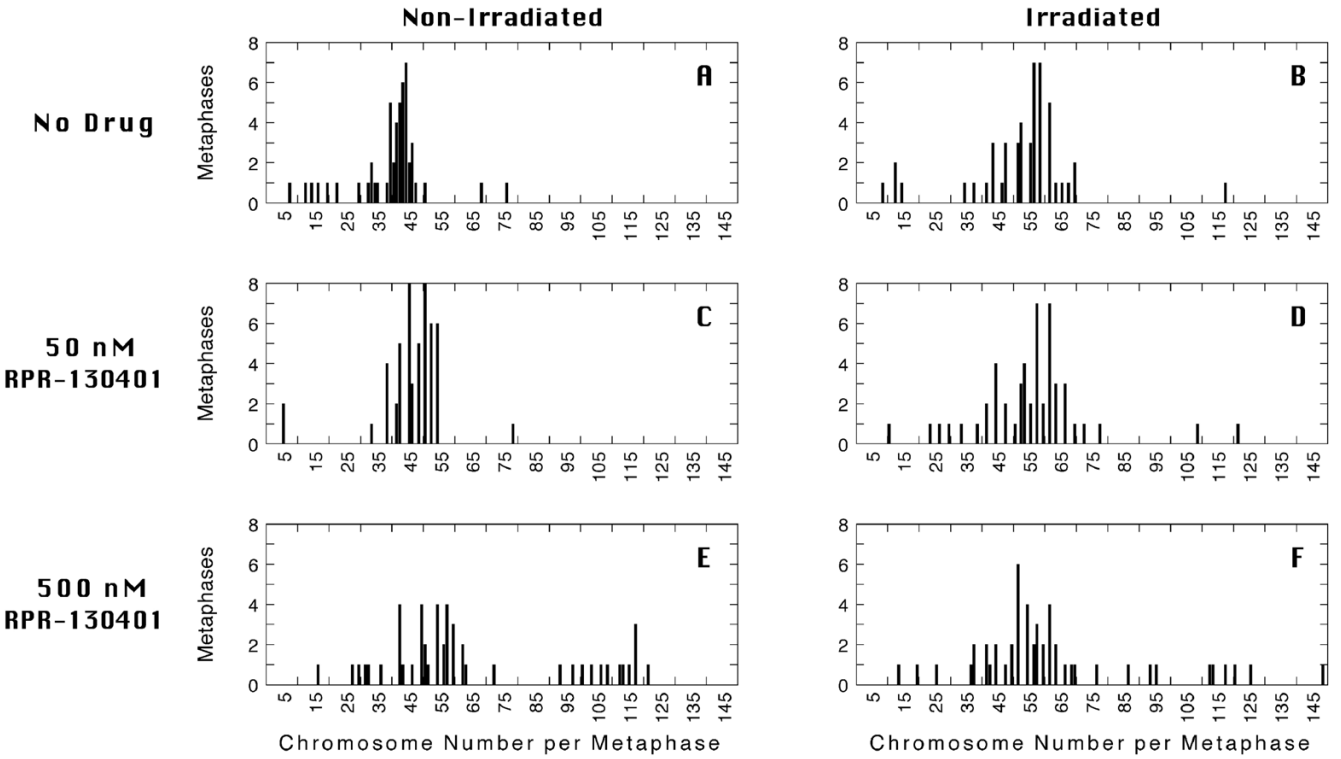


Figure 7
Effect of RPR-130401 exposure on the chromosome number distribution in individual metaphases. HCT-116 cells were grown for 4-days without (A, B) or with 50 nM (C, D) or 500 nM (E, F) RPR-130401. Cells were then given 3.5 Gy γ-rays (B, D, F) and returned to the incubator for 24-h prior to fixation and scoring. Each panel represents the counts from at least 50 metaphases.

tubule and actin networks, even after prolonged incubation with a high drug concentration in the most sensitive cell line (500 nM, HCT-116 cells); *(vi)* strictly additive interaction with ionizing radiation with regard to cell kill, cell cycle redistribution and induction of hyperploidy.

Two alternative pathways should be taken into consideration in order to explain these observations, namely, inhibition of the farnesylation of $p21^{Ras}$ or related proteins from the Ras superfamily, or inhibition of the farnesylation of other proteins resulting in disruption of the mitotic apparatus or cytokinesis traverse. These hypotheses are discussed below.

$p21^{Ras}$ activation in normal and tumor cells is mandatory to the transition from cell quiescence to proliferation. However, it is generally agreed that inhibition of $p21^{Ras}$ farnesylation does not suppress mitogenic functions in Ras-transformed cells [38]. Moreover, it has recently been shown that growth inhibition by FTIs does not necessarily proceed from inhibition of $p21^{Ras}$ prenylation, and that various Ras-unrelated proteins may be important targets for FTI treatment [2,19,29,39,40]. Even though it results in an altered subcellular distribution of $p21^{Ras}$[6], RPR-130401 is not able to block $p21^{Ras}$ geranylgeranylation [24] in such a way that inhibition of $p21^{Ras}$ farnesylation is not likely to account for the main effects of the drug. On the other hand, the peptidomimetic FTI L-739,749 has been reported to prevent Ras-induced transformation through inhibition of Rho-B prenylation [31] with a block in cell proliferation, increased apoptosis [41] and major changes in stress fiber formation, cell shape and mobility. None of these characteristic changes in cell morphology and architecture was observed upon prolonged exposure to RPR-130401 (Figure 6), which seems to rule out any member of the Rho family as a target for RPR-130401.

Considering the efficient pre-mitotic block and hyperploidy induced by RPR-130401, as also found with other FTIs [16,29,30,42], it is tempting to postulate that the target of the drug is a protein involved at some stage in mitosis and/or cytokinesis, and whose farnesylation is required for proper activation. Indeed, protein isoprenylation is important at the G2/M transition in *Schizosaccharomyces pombe*[43] and nuclear lamins are major targets for FTase [18,44]. Lamin B farnesylation, which is a very sensitive target for RPR-130401 *in vitro*[6], appears to deserve con-

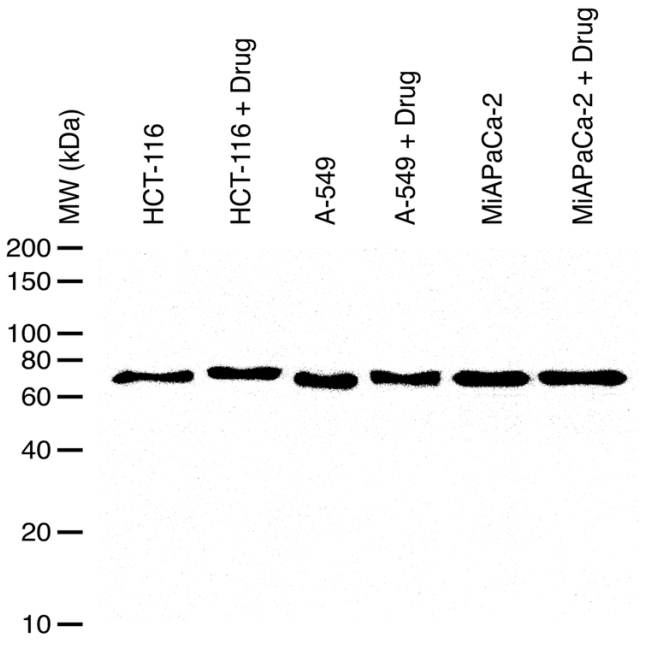


Figure 8
Western blot analysis of lamin B in extracts from control and treated cells. Cells were incubated for 5-days in 75 cm^2 flasks with 500 nM (HCT-116), 7.1 μM (A-549) or 1.2 μM (MiA-PaCa-2) RPR-130401. A blank was also prepared for each cell line. The number of cells at the time of cell lysis, was in the range 10^7. At the end of treatment, the flasks were rinsed twice with ice-cold PBS and cells lysed in PHEM buffer as described under Materials and Methods. An aliquot of each extract (20 μg total protein) was loaded in the wells of a 7.5% polyacrylamide gel and electrophoresis was performed under usual conditions with molecular weight standards, followed by transfer to a nitrocellulose membrane. The membrane was incubated with a mouse monoclonal antibody directed against human lamin B and revealed through chemiluminescence detection (see Materials and Methods).

sideration as a candidate to this role. As a matter of fact, among the three major type A-C lamins, lamin A and lamin B are initially translated with a CAAX motif. This motif is cleaved upon post-translational maturation of lamin A but persists in lamin B, unless the C-terminal aminoacid residue is mutated [23]. Studies with mutant lamin deleted of the CAAX sequence have shown that the CAAX motif is essential to anchoring lamin B to the nuclear envelope [45,46]; moreover, farnesylation is required for the integration of newly synthesized lamins into the pre-existing nuclear lamina during interphase, and GGTase is unable to substitute for FTase at this stage [47].

These data altogether suggest that inhibition of lamin B farnesylation could be an important target of RPR-130401, resulting in an aberrant onset of mitosis and cytokinesis and ensuing hyperploid cell generation. Strong support to this scheme is given by immunofluorescence experiments showing that the intranuclear distribution of lamin B is profoundly altered in cells exposed to RPR-130401 (Figure 5). This occurred without lamin B breakdown (Figure 8), consistent with lack of a significant level of apoptosis in the three cell lines used. Interestingly SCH-66336, another FTI, reportedly allows accumulation of unfarnesylated prelamin A in patients under phase I investigation [1] and in several cell lines in culture as well [48]. It has also been shown that SCH-66336 induces diruption of microtubule binding to the centromeric proteins CENP-E and CENP-F [17], thus evoking again faulty mitosis as an important outcome of FTI exposure.

Conclusions

Oncogenic *Ras* expression has been reported to have different outcomes in rodent and human cells with regard to radiation susceptibility. Studies in mouse [49–52] or rat cells [51,53–59] relied on transfection with *N-Ras*[49,50], *H-Ras*[51,53–58] or co-transfection with *H-Ras* and *myc*[53–55], [58–60]. Except in one study [51], the authors have conclusively shown that transformation of rodent cells with the *Ras* oncogenes results in enhanced radiation resistance. Co-transfection with *H-Ras* and *myc* was found to act synergistically with regard to induction of the radioresistant phenotype [53,54,58–60], possibly in relation to inhibition of *myc*-dependent apoptosis [58,61].

With two exceptions in support of increased radioresistance [62,63], early studies on human cells did not show any clear-cut relationship between radiation sensitivity and expression of oncogenic *Ras*[64–67]. In addition, a study involving a cohort of patients with stage II and III carcinoma of the uterine cervix, demonstrated the absence of a significant correlation between rearrangements or structure of the *Ha-ras-1* and/or *c-myc* genes and tumor response to radiotherapy [68]. However, the contribution of *N- or K-Ras* activation to intrinsic radioresistance in human cells has recently been adressed directly, i.e., without the perturbations introduced by cell transformation [69]. The mechanisms involved in *Ras*-related radioresistance are still open to question, but it might reasonably be proposed that it proceeds from constitutive activation of pathways dowstream from *Ras* in signal transduction, such as raf-1 [70] or phosphatidylinositol 3-kinase [71].

Whether and how FTase inhibition may affect the response of cells to ionizing radiation has also been a long-standing problem. The outcome appears to depend very much on the cell line and the specificity of the FTI used. The FTase inhibitor FTI-277 reportedly acted as a radiosensitizer in *H-Ras*-transformed rat embryo fibroblasts [72] and in human tumor cells harboring *H-Ras* mutation [73]. On the other hand, the radiation susceptibility of human tumor cells expressing the wild-type form of *Ras*

was not altered by FTIs [73]. However, enhanced radiation response by FTI-277 has been described in human cells expressing wild-type *Ras* together with the 24-kDa isoform (FGF2) of basic fibroblast growth factor, a factor involved in acquired radioresistance [74]. This was taken as an indication that farnesylated proteins other than Ras might be involved in the modulation of radiation response by FTI-277 [74]. Weak radiosensitization only was obtained with FTI-277 in K-Ras mutants, unless FTI-277 was combined with GGTI-298 to hinder geranylgeranyl transfer to K-Ras [73]. Last but not least FTI-induced radiosensitization, when it occurs, seems to be independent on the growth inhibitory effect of the FTI [73].

We show here that RPR-130401 does not significantly alter the radiation susceptibility of human cells bearing K-Ras mutation. This occurs in spite of significant hyperploidization, accumulation of cells in G2 and depletion of S phase. Finally, impaired repair of radiation damage by RPR-130401, appears to be very unlikely to occur. This seems valuable in the prospect of combining RPR-130401 as an adjuvant to conventional radiotherapy, as it should provide strictly additive interaction without the local and systemic adverse effects inherent in treatments with radiation sensitizers [75]. In this respect it is very exciting to note that two farnesyltransferase inhibitors, FTI-276 and L-744,832, have recently proven to act synergistically with ionizing radiation *in vivo* for growth inhibition of xenotransplanted *H-Ras*-mutated human tumor cells, without increase in acute or long-term radiation-induced damage to healthy tissues [76].

Materials and methods

Reagents

RPR-130401 was synthesized as described [77] and stored at -20°C as a 20 mM stock solution in DMSO. Prior to use, the solution was diluted first into medium containing 20% DMSO, then to the final concentration in culture medium. DMSO was kept below 0.1% so as not to alter cell growth or radiation response.

Colcemid and all products from cell culture were from Invitrogen (Cergy-Pontoise, France). BrdUrd, propidium iodide, bisbenzimide, DAPI, Giemsa stain, TRITC-phalloidin and mouse anti-α-tubulin monoclonal antibody (clone DM1A) came from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Mouse monoclonal antibodies directed against lamin B was from Oncogene Research Products (San Diego, California). FITC-conjugated sheep anti-mouse IgG was from Sanofi-Synthélabo (Paris, France). Alexa® 488 goat anti-mouse IgG conjugate was from Molecular Probes (Eugene, Oregon). Rat monoclonal antibody directed against BrdUrd was purchased from Harlan Sera-Lab (Loughborough, United Kingdom), and fluorescein isothiocyanate-conjugated goat anti-rat IgG (heavy chain specific) was from Southern Biotechnology Associates (Birmingham, Alabama). Human centromere probes for FISH was obtained from QBiogene (Illkirch, France). Solvents and chemicals were of the highest purity available and came from Merck Eurolab (Paris, France).

Cell culture

HCT-116 human colon carcinoma (ATCC CCL-247), MiA-PaCa-2 human pancreatic carcinoma (ATCC CRL-1420) and A-549 human lung carcinoma cells (ATCC CCL-185) were grown as monolayers in DMEM (HCT-116, MiAPaCa-2) or RPMI-1640 (A-549 cells) containing sodium pyruvate and Glutamax I, and supplemented with 10% foetal calf serum, 100 IU/ml penicillin, and 0.1 mg/ml streptomycin. Cells were routinely subcultured after trypsinization at a density of 6,000 cells/cm^2 in culture flasks every 5-days and incubated at 37°C in a humidified atmosphere of 7% CO_2 in air. Less than 9–10 passages of the same primary subculture were allowed. Prior to seeding, cells were rinsed twice with HBSS, harvested by trypsin-EDTA treatment and counted in an hemocytometer.

Survival assays

Radiation survival with or without combined drug exposure was determined by clonogenic assays. For determination of the response to radiation alone, cells from exponentially growing subcultures were seeded in 25 cm^2 flasks at the suitable density, incubated for 3-h to allow cells to adhere and spread, and irradiated in culture medium at room temperature in an IBL-637 (^{137}Cs) irradiator (CIS-Biointernational, Saclay, France) at a dose-rate of 0.92 Gy/min. All experiments were performed in triplicate or more.

Two protocols were used in single or combined treatment assays. For short lengths of drug exposure (≤ 12-h), 1,000 cells were plated in triplicate in 25 cm^2 flasks and incubated for 3-h. Cells were then exposed to drug for 1-, 2-, 3-, 4- or 12-h, irradiated and returned to the incubator for 1-h in the presence of RPR-130401, followed by two washes with HBSS and feeding with drug-free medium. Colonies were allowed to grow for 8–11 days, then fixed with methanol, stained with Giemsa and scored. Small colonies (< 30–50 cells) were disregarded. Results were corrected for cell multiplicity (see below).

Where the length of drug exposure was in excess of 12-h, cells were plated after treatments to avoid biases due to the formation of microcolonies. Typically, cells were plated in 25 cm^2 flasks and exposed to drug for 1-, 2-, 3-, 4- or 5-days, and irradiated. Seeding was adjusted so as to obtain an equal number of cells at the time of irradiation, taking into consideration the cytostatic effect of the FTI and the length of drug exposure. Each flask was

trypsinized immediately after irradiation, the cells were counted and 1,000 cells per flask were replated in drug-free medium for colony formation assays.

Flow cytometry and cytogenetic analysis

Cell cycle progression was monitored by dual parameter flow cytometry using a FACStar PLUS cytofluorometer (Becton Dickinson, Le Pont de Claix, France). Cells were grown for 3- to 6-days with or without RPR-130401. At that time, flasks were irradiated to 3.5 Gy (HCT-116), 5 Gy (A-549) or 5.5 Gy (MiAPaCa-2) and allowed to rest in the incubator for 24-h prior to fixation. 15-min before harvesting, cells were incubated with BrdUrd (10 μM) for pulse-labeling of S-phase cells, then collected, washed once with cold PBS and fixed in 70% ice-cold ethanol. Treatment of fixed cells for cytofluorimetric analysis, data acquisition and processing were done according to Demarcq *et al.*[78]. Cell cycle analysis was performed with ProCyt software (CEA-INSERM, Grenoble, France).

Alternatively, HCT-116 treated cells were incubated with 0.2 μg/ml colcemid for 1-h, washed twice with HBSS and allowed to swell for 30-min in 75 mM KCl hypotonic solution. Cells were then fixed in 3:1 v/v methanol:acid acetic in ice and washed threefold in the same solvent. Fixed cells were dropped onto glass slides to spread chromosomes, and air dried. Slides were stained with Giemsa before scoring.

Immunofluorescence

For immunofluorescence characterization, cells were grown on 20 × 20 mm coverslips in medium containing 10% foetal calf serum for 3- to 6-days with or without RPR-130401. At that time, the coverslips were irradiated to 3.5 Gy (HCT-116), 5 Gy (A-549) or 5.5 Gy (MiAPaCa-2) and allowed to rest in the incubator for 24-h prior to fixation.

To visualize polymerized actin and tubulin, cells were processed according to Mies *et al.*[79]. Briefly, cells were fixed in 0.25% glutaraldehyde, 0.5% Triton X-100 for 1-min, followed by 10-min in freshly prepared 4% formaldehyde and extensive washing. The coverslips were then incubated (30-min, 37°C) with 0.2 μg/ml TRITC-labeled phalloidin, or with a 1:200 working dilution of monoclonal anti-α tubulin antibody followed by a 30-min incubation with a 1:1,000 dilution of FITC-conjugated sheep anti-mouse IgG at 37°C.

To visualize lamin, cells were fixed in acetone/methanol (1:1, v/v) for 20-min at -20°C, allowed to air dry, then washed 3 times with PBS and treated with 0.5% Triton X-100 for 5-min at 4°C to permeabilize cells. The coverslips were incubated (30-min, 37°C) with 5 μg/ml of monoclonal anti-lamin B antibody followed by a 30-min incubation with 1:500 Alexa® 488 goat anti-mouse IgG conjugate.

To visualize nuclei, the cells were counter-stained with bisbenzimide (0.2 μg/ml) or DAPI (0.12 μg/ml). Cells were viewed on a Zeiss microscope and photographed with Ilford HP-5 film.

Western blots

For determination of lamin B cleavage, HCT-116, A-549 and MiAPaCa-2 cells were exposed for 5-days at $10 \times IC_{50}$ each of RPR-130401. At the end of treatment, cells were harvested in PBS with 2 mM EDTA and lysed for 20-min on ice with gentle agitation in PHEM buffer (80 mM PIPES, 80 mM HEPES, 10 mM EDTA, 5 mM $MgCl_2$, 1% Triton X-100, pH 7.4) supplemented with 1 mM PMSF, 10 μg/ml aprotinin and 1 μg/ml each of leupeptin and pepstatin. The insoluble fraction, containing lamin B, was recovered by centrifugation, solubilized in Laemmli buffer (3% lauryl sulfate, 10% glycerol, 5% 2-mercaptoethanol, 62.5 mM Tris-HCl, pH 6.8) and titrated.

An aliquot (20 μg total protein) of each extract was loaded onto 7.5% polyacrylamide gel, electrophoresed, and blotted onto nitrocellulose (Schleicher & Schuell, Dassel, Germany). The membrane was saturated with bovine serum albumine and incubated with a mouse monoclonal antibody directed against lamin B. Protein detection was carried out using a peroxidase-conjugated goat anti-mouse secondary antibody and an ECL kit (Amersham Pharmacia Biotech, Orsay, France). Quantification was performed by densitometry with the aid of QuantityOne® software (Bio-Rad, Hercules, California).

Curve fittings and correction for cellular multiplicity

Care was taken to avoid contamination of seed cell suspensions by clusters, as their presence introduces large, systematic errors in viability measurements with clonogenic assays [80]. For this reason, the cellular multiplicity, *i.e.*, the number of cells per potential colony-forming unit was carefully checked by microscope observation at the time of irradiation throughout the whole study. When the average multiplicity was less than 1.1 (for 1- and 2-h drug exposure), no correction was applied. Corrections were necessary for longer lengths of drug exposure (≥ 3-h), because viable colony-forming units contained a substantial amount of doublets and quadruplets at these times. In that case, the equation:

$$(1) \qquad S_{exp} = \sum_{1}^{n} a_n \left[1 - \left(1 - SCSF \right)^n \right]$$

(where S_{exp} is the experimental cell survival determined from bulk colony scoring and a_n the fraction of colony-

forming units with n viable cells) was used in order to calculate the single-cell surviving fraction (SCSF).

Radiation survival curves with or without co-treatment with RPR-130401 were drawn for best fit to a linear-quadratic equation, as usual [81]:

$$(2) \qquad \ln\frac{S}{S_0} = -\alpha D - \beta D^2$$

where S_0 is the clonogenic efficiency, S the residual survival, D the radiation dose, and α and β numerical parameters characterizing the radiosensitivity of the cell line.

Equation (1) was solved with the aid of a home-made program. Least-squares regressions were performed on a Macintosh microcomputer using Kaleidagraph software (Synergy Software, Reading, Pennsylvania).

List of abbreviations

FTase, farnesyltransferase;

FPP, farnesyl pyrophosphate;

FTI, farnesyltransferase inhibitor;

GGTase, geranylgeranyl transferase; HBSS, Hank's balanced salt solution;

BrdUrd, 5-Bromo-2'-deoxyuridine;

PMSF, phenylmethylsulfonyl fluoride;

FISH, Fluorescence In Situ Hybridation; IC_{50}, amount of drug that reduces the growth of treated cells to 50% of that of controls.

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References

1. Adjei AA, Erlichman C, Davis JN, Cutler DL, Sloan JA, Marks RS, Hanson LJ, Svingen PA, Atherton P, Bishop WR, Kirschmeier P, Kaufmann SH: **A Phase I trial of the farnesyl transferase inhibitor SCH66336: evidence for biological and clinical activity.** *Cancer Res* 2000, **60:**1871-1877
2. Crul M, de Klerk GJ, Beijnen JH, Schellens JHM: **Ras biochemistry and farnesyl transferase inhibitors: a literature survey.** *Anticancer Drugs* 2001, **12:**163-184
3. Karp JE, Lancet JE, Kaufmann SH, End DW, Wright JJ, Bol K, Horak I, Tidwell ML, Liesveld J, Kottke TJ, Ange D, Buddharaju L, Gojo I, Highsmith WE, Belly RT, Hohl RJ, Rybak ME, Thibault A, Rosenblatt J: **Clinical and biologic activity of the farnesyltransferase inhibitor R115777 in adults with refractory and relapsed acute leukemias: a phase 1 clinical-laboratory correlative trial.** *Blood* 2001, **97:**3361-3369
4. Punt CJ, van Maanen L, Bol CJ, Seifert WF, Wagener DJ: **Phase I and pharmacokinetic study of the orally administered farnesyl transferase inhibitor R115777 in patients with advanced solid tumors.** *Anticancer Drugs* 2001, **12:**193-197
5. Zujewski J, Horak ID, Bol CJ, Woestenborghs R, Bowden C, End DW, Piotrovsky VK, Chiao J, Belly RT, Todd A, Kopp WC, Kohler DR, Chow C, Noone M, Hakim FT, Larkin G, Gress RE, Nussenblatt RB, Kremer AB, Cowan KH: **Phase I and pharmacokinetic study of farnesyl protein transferase inhibitor R115777 in advanced cancer.** *J Clin Oncol* 2000, **18:**927-941
6. Mailliet P, Riou J-F, Duchesne M, Lelièvre Y, Lavayre J, Bourzat J-D, Capet M, Chevé M, Commerçon A, Martin M, Thompson F, Dereu N, Lavelle F: **Benzo(f)perhydroisoindoles: a series of potent and selective inhibitors of the farnesylation of Ki-Ras.** *Proc Amer Assoc Cancer Res* 1998, **39:**270
7. Bos JL: **Ras oncogenes in human cancer: a review.** *Cancer Res* 1989, **49:**4682-4689
8. Marshall CJ: **Protein-prenylation: a mediator of protein-protein interactions.** *Science* 1993, **259:**1865-1866
9. Zhang FL, Casey PJ: **Protein prenylation: molecular mechanisms and functional consequences.** *Annu Rev Biochem* 1996, **65:**241-269
10. James GL, Goldstein JL, Brown MS: **Polylysine and CVIM sequences of K-RasB dictate specificity of prenylation and confer resistance to benzodiazepine peptidomimetic *in vitro*.** *J Biol Chem* 1995, **270:**6221-6226
11. Jackson JH, Cochrane CG, Bourne JR, Solski PA, Buss JE, Der CJ: **Farnesol modification of Kirsten-ras 4B protein is essential for transformation.** *Proc Natl Acad Sci USA* 1990, **87:**3042-3046
12. Kato K, Cox AD, Hisaka MM, Graham SM, Buss JE, Der CJ: **Isoprenoid addition to ras protein is the critical modification for its membrane association and transforming activity.** *Proc Natl Acad Sci USA* 1992, **89:**6403-6407
13. Lowy DR, Willumsen BM: **Function and regulation of ras.** *Annu Rev Biochem* 1993, **62:**851-891
14. Gibbs JB, Oliff A, Kohl NE: **Farnesyltransferase inhibitors: ras research yields a potential cancer therapeutic.** *Cell* 1994, **77:**175-178
15. Tamanoi F: **Inhibitors of ras farnesyltransferases.** *Trends Biochem Sci* 1993, **18:**349-353
16. Miquel K, Pradines A, Sun J, Qian Y, Hamilton AD, Sebti SM, Favre G: **GGTI-298 induces G0-G1 block and apoptosis whereas FTI-277 causes G2-M enrichment in A549 cells.** *Cancer Res* 1997, **57:**1846-1850
17. Ashar HR, James L, Gray K, Carr D, Black S, Armstrong L, Bishop WR, Kirschmeier P: **Farnesyl transferase inhibitors block the farnesylation of CENP-E and CENP-F, and alter the association of CENP-E with the microtubules.** *J Biol Chem* 2000, **275:**30451-30457
18. Sinensky M: **Recent advances in the study of prenylated proteins.** *Biochim Biophys Acta* 2000, **1484:**93-106
19. Cox AD: **Farnesyltransferase inhibitors: potential role in the treatment of cancer.** *Drugs* 2001, **61:**723-732
20. Gibbs JB: **Ras C-terminal processing enzymes-new drug targets?** *Cell* 1991, **65:**1-4
21. Glomset JA, Gelb MH, Farnsworth CC: **Prenyl proteins in eukaryotic cells: a new type of membrane anchor.** *Trends Biochem Sci* 1990, **15:**139-142
22. Vrignaud P, Bello A, Bissery MC, Jenkins R, Hasnain A, Mailliet P, Lavelle F: **RPR130401 a non-peptidomimetic farnesyltransferase inhibitor with *in vivo* activity.** *Proc Amer Assoc Cancer Res* 1998, **39:**270
23. Yokoyama K, Goodwin GW, Gomashchi F, Glomset JA, Gelb MH: **A protein geranylgeranyltransferase from bovine brain: implications for protein prenylation specificity.** *Proc Natl Acad Sci USA* 1991, **88:**5302-5306
24. Mazet JL, Padieu M, Osman H, Maume G, Mailliet P, Dereu N, Hamilton AD, Lavelle F, Sebti SM, Maume BF: **Combination of the novel farnesyltransferase inhibitor RPR130401 and the geranylgeranyltransferase-I inhibitor GGTI-298 disrupts MAP kinase activation and G(1)-S transition in Ki-Ras-overexpressing transformed adrenocortical cells.** *FEBS Lett* 1999, **460:**235-240

25. Cates CA, Michael RL, Stayrook KR, Harvey KA, Burke YD, Randall SK, Crowell PL, Crowell DN: **Prenylation of oncogenic human PTP(CAAX) protein tyrosine phosphatases.** *Cancer Lett* 1996, **110:**49-55
26. Shirasawa S, Furuse M, Yokoyama N, Sasazuki T: **Altered growth of human colon cancer cell lines disrupted at activated Ki-ras.** *Science* 1993, **260:**85-88
27. Berrozpe G, Schaeffer J, Peinado MA, Real FX, Perucho M: **Comparative analysis of mutations in the p53 and K-ras genes in pancreatic cancer.** *Int J Cancer* 1994, **58:**185-191
28. Valenzuela DM, Groffen J: **Four human carcinoma cell lines with novel mutations in position 12 of c-K-ras oncogene.** *Nucleic Acids Res* 1986, **14:**843-852
29. Song SY, Meszoely IM, Coffey RJ, Pietenpol JA, Leach SD: **K-Ras-independent effects of the farnesyl transferase inhibitor L-744,832 on cyclin B1/Cdc2 kinase activity, G2/M cell cycle progression and apoptosis in human pancreatic ductal adenocarcinoma cells.** *Neoplasia* 2000, **2:**261-272
30. Crespo NC, Ohkanda J, Yen TJ, Hamilton AD, Sebti SM: **The farnesyltransferase inhibitor, FTI-blocks bipolar spindle formation and chromosome alignment and causes prometaphase accumulation during mitosis of human lung cancer cells.** *J Biol Chem* 2153, **276:**16161-16167
31. Lebowitz PF, Davide JP, Prendergast GC: **Evidence that farnesyltransferase inhibitors suppress Ras transformation by interfering with Rho activity.** *Mol Cell Biol* 1995, **15:**6613-6622
32. Prendergast GC: **Farnesyltransferase inhibitors: antineoplastic mechanism and clinical prospect.** *Curr Opin cell Biol* 2000, **12:**166-173
33. Prendergast GC, Davide JP, de Solms SJ, Giuliani EA, Graham SL, Gibbs JB, Oliff A, Kohl NE: **Farnesyltransferase inhibition causes morphological reversion of ras-transformed cells by a complex mechanism that involves regulation of the actin cytoskeleton.** *Mol Cell Biol* 1994, **14:**4193-4202
34. Denko NC, Giaccia AJ, Stringer JR, Stambrook PJ: **The human Ha-ras oncogene induces genomic instability in murine fibroblasts within one cell cycle.** *Proc Natl Acad Sci USA* 1994, **91:**5124-5128
35. Denko NC, Stringer J, Wani M, Stambrook PJ: **Mitotic and post mitotic consequences of genomic instability induced by oncogenic Ha-Ras.** *Somatic Cell Mol Genet* 1995, **21:**241-253
36. Kowluru A: **Evidence for the carboxyl methylation of nuclear lamin-B in the pancreatic beta cell.** *Biochem Biophys Res Commun* 2000, **268:**249-254
37. Oberhammer FA, Hochegger K, Froschl G, Tiefenbacher R, Pavelka M: **Chromatin condensation during apoptosis is accompanied by degradation of lamin A+B, without enhanced activation of cdc2 kinase.** *J Cell Biol* 1994, **126:**827-837
38. Kohl NE, Mosser SD, de Solms SJ, Giuliani EA, Pompliano DL, Graham SL, Smith RL, Scolnick EM, Oliff A, Gibbs JB: **Selective inhibition of ras-dependent transformation by a farnesyltransferase inhibitor.** *Science* 1993, **260:**1934-1397
39. Lebowitz PF, Prendergast GC: **Non-Ras targets of farnesyltransferase inhibitors: focus on Rho.** *Oncogene* 1998, **17:**1439-1445
40. Servais P, Gulbis B, Fokan D, Galand P: **Effects of the farnesyltransferase inhibitor UCF-1C/Manumycin on growth and p21-Ras post-translational processing in NIH3T3 cells.** *Int J Cancer* 1998, **76:**601-608
41. Du W, Lebowitz F, Prendergast GC: **Cell growth inhibition of farnesyltransferase inhibitors is mediated by gain of geranylgeranylated RhoB.** *Mol Cell Biol* 1999, **19:**1831-1840
42. Ashar HR, James L, Gray K, Carr D, McGuirk M, Maxwell E, Black S, Armstrong L, Doll RJ, Taveras AG, Bishop WR, Kirschmeier P: **The farnesyl transferase inhibitor SCH 66336 induces a G(2) → M or G(1) pause in sensitive human tumor cell lines.** *Exp Cell Res* 2001, **262:**17-27
43. Galli I, Uchiyama M, Wang TS: **DNA replication and order of cell cycle events: a role for protein isoprenylation?** *Biol Chem* 1997, **378:**963-973
44. Farnsworth CC, Wolda SL, Gelb MH, Glomset JA: **Human lamin B contains a farnesylated cysteine residue.** *J Biol Chem* 1989, **264:**20422-20429
45. Kitten GT, Nigg EA: **The CAAX motif is required for isoprenylation, carboxyl methylation, and nuclear membrane association of lamin B2.** *J Cell Biol* 1991, **113:**13-23
46. Mical TI, Monteiro MJ: **The role of sequences unique to nuclear intermediate filaments in the targeting and assembly of human lamin B: evidence for lack of interaction of lamin B with its putative receptor.** *J Cell Sci* 1998, **111:**3471-3485
47. Hennekes H, Nigg EA: **The role of isoprenylation in membrane attachment of nuclear lamins : a single point mutation prevents proteolytic cleavage of the lamin A precursor and confers membrane binding properties.** *J Cell Sci* 1994, **107:**1019-1029
48. Adjei AA, Davis JN, Erlichman C, Svingen PA, Kaufmann SH: **Comparison of potential markers of farnesyltransferase inhibition.** *Clin Cancer Res* 2000, **6:**2318-2325
49. Fitzgerald TJ, Daugherty C, Kase K, Rothstein LA, McKenna M, Greenberger JS: **Activated human N-ras oncogene enhances X-irradiation repair of mammalian cells *in vitro* less effectively at low dose rate. Implications for increased therapeutic ratio of low dose rate irradiation.** *Am J Clin Oncol* 1985, **8:**517-522
50. Sklar MD: **The ras oncogenes increase the intrinsic resistance of NIH3T3 cells to ionizing radiation.** *Science* 1988, **239:**645-647
51. Harris JF, Chambers AF, Tam ASK: **Some *ras*-transformed cells have increased radiosensitivity and decreased repair of sublethal radiation damage.** *Somatic Cell Mol Genet* 1990, **16:**39-48
52. Pirollo KF, Tong Y-A, Villegas Z, Chen Y, Chang EH: **Oncogene-transformed NIH3T3 cells display radiation resistance levels indicative of a signal transduction pathway leading to the radiation-resistant phenotype.** *Radiat Res* 1993, **135:**234-243
53. McKenna WG, Weiss MC, Bakanauskas VJ, Sandler H, Kelsten ML, Biaglow J, Tuttle SW, Endlich B, Ling CC, Muschel RJ: **The role of the *H-ras* oncogene in radiation resistance and metastasis.** *Int J Radiat Oncol Biol Phys* 1990, **18:**849-859
54. McKenna WG, Weiss MC, Endlich B, Ling CC, Bakanauskas VJ, Kelsten ML, Muschel RJ: **Synergistic effect of the *v-myc* oncogene with *H-ras* radioresistance.** *Cancer Res* 1990, **50:**97-102
55. Iliakis G, Metzger L, Muschel RJ, McKenna WG: **Induction and repair of DNA double strand breaks in radiation-resistant cells obtained by transformation of primary rat embryo cells with the oncogenes *H-ras* and *v-myc*.** *Cancer Res* 1990, **50:**6575-6579
56. Hermens AF, Bentvelzen PAJ: **Influence of the *H-ras* oncogene on radiation responses of a rat rhabdomyosarcoma cell line.** *Cancer Res* 1992, **52:**3073-3082
57. Ong A, Li WX, Ling CC: **Low-dose-rate irradiation of rat embryo cells containing the *Ha-ras* oncogene.** *Radiat Res* 1993, **134:**251-255
58. McKenna WG, Bernhard EJ, Markiewicz DA, Rudoltz MS, Maity A, Muschel RJ: **Regulation of radiation-induced apoptosis in oncogene-transfected fibroblasts: influence of *H-ras* on the G2 delay.** *Oncogene* 1996, **12:**237-245
59. Ling CC, Endlich B: **Radioresistance induced by oncogenic transformation.** *Radiat Res* 1989, **120:**267-279
60. McKenna WG, Iliakis G, Weiss MC, Bernhard EJ, Muschel RJ: **Increased G2 delay in radiation-resistant cells obtained by transformation of primary rat embryo cells with the oncogenes *H-ras* and *v-myc*.** *Radiat Res* 1991, **125:**283-287
61. Chen CH, Zhang J, Ling CC: **Transfected *c-myc* and *c-Ha-ras* modulate radiation-induced apoptosis in rat embryo cells.** *Radiat Res* 1994, **139:**307-315
62. Miller AC, Kariko K, Myers CE, Clark EP, Samid D: **Increased radioresistance of EJ-ras-transformed human osteosarcoma cells and its modulation by lovastatin, an inhibitor of p21ras isoprenylation.** *Int J Cancer* 1993, **53:**302-307
63. Bruyneel EA, Storme GA, Schallier DCC, Van den Berge DL, Hilgard P, Mareel MM: **Evidence for abrogation of oncogene-induced radioresistance of mammary cancer cells by hexadecylphosphocholine *in vitro* .** *Eur J Cancer* 1993, **29A:**1958-1963
64. Grant ML, Bruton RK, Byrd J, Gallimore PH, Steele JC, Taylor AML, Grand RJA: **Sensitivity to ionising radiation of transformed human cells containing mutant *ras* genes.** *Oncogene* 1990, **5:**1159-1164
65. Alapetite C, Baroche C, Remvikos Y, Goubin G, Moustacchi E: **Studies on the influence of the presence of an activated ras oncogene on the *in vitro* radiosensitivity of human mammary epithelial cells.** *Int J Radiat Biol* 1991, **59:**385-396
66. Mendonca MS, Boukamp P, Stanbridge EJ, Redpath JL: **The radiosensitivity of human keratinocytes: influence of activated c-H-ras oncogene and tumorigenicity.** *Int J Radiat Biol* 1991, **59:**1195-1206

67. Minarik L, Hall E, Miller R: **Tumorigenicity, oncogene transfection, and radiosensitivity.** *Cancer J Sci Am* 1996, **2:**351
68. Polischouk AG, Scotnikova OI, Sergeeva NS, Zharinov GM, Lewensohn R, Zhivotovsky B: **Response to radiotherapy of human uterine cervix carcinoma is not correlated with rearrangements of the Ha-ras-1 and/or c-myc genes.** *Eur J Cancer* 1997, **33:**942-949
69. Bernhard EJ, Stanbridge EJ, Gupta S, Gupta AK, Soto D, Bakanauskas VJ, Cerniglia GJ, Muschel RJ, McKenna WG: **Direct evidence for the contribution of activated N-ras and K-ras oncogenes to increased intrinsic radiation resistance in human tumor cell lines.** *Cancer Res* 2000, **60:**6597-6600
70. Pirollo KF, Hao Z, Rait A, Ho CW, Chang EH: **Evidence supporting a signal transduction pathway leading to the radiation-resistant phenotype in human tumor cells.** *Biochem Biophys Res Commun* 1997, **230:**196-201
71. Gupta AK, Bakanauskas VJ, Cerniglia GJ, Cheng Y, Bernhard EJ, Muschel RJ, McKenna WG: **The Ras radiation resistance pathway.** *Cancer Res* 2001, **61:**4278-4282
72. Bernhard EJ, Kao G, Cox AD, Sebti SM, Hamilton AD, Muschel RJ, McKenna WG: **The farnesyltransferase inhibitor FTI-277 radiosensitizes *H-ras*-transformed rat embryo fibroblasts.** *Cancer Res* 1996, **56:**1727-1730
73. Bernhard EJ, McKenna WG, Hamilton AD, Sebti SM, Qian Y, Wu J-M, Muschel RJ: **Inhibiting ras prenylation increases the radiosensitivity of human tumor cell lines with activating mutations of *ras* oncogenes.** *Cancer Res* 1998, **58:**1754-1761
74. Cohen-Jonathan E, Toulas C, Ader I, Monteil S, Allal C, Bonnet J, Hamilton AD, Sebti SM, Daly-Schveitzer N, Favre G: **The farnesyltransferase inhibitor FTI-277 suppresses the 24-kDa FGF2-induced radioresistance in HeLa cells expressing wild-type RAS.** *Radiat Res* 1999, **152:**404-411
75. Balosso J, Minne J-F, Touboul E: **Late complications of chemoradiotherapeutic combinations: fundamental aspects and clinical experience.** *Bull Cancer Radiother* 1995, **82:**101-112
76. Cohen-Jonathan E, Muschel RJ, McKenna WG, Evans SM, Cerniglia G, Mick R, Kusewitt D, Sebti SM, Hamilton AD, Oliff A, Kohl N, Gibbs JB, Bernhard EJ: **Farnesyltransferase inhibitors potentiate the antitumor effect of radiation on a human tumor xenograft expressing activated HRAS.** *Radiat Res* 2000, **154:**125-132
77. Mailliet P, Laoui A, Bourzat J-D, Capet M, Chevé M, Commerçon A, Dereu N, LeBrun A, Martin J-P, Peyronel J-F, Salagnad C, Thompson F, Zucco M, Guitton J-D, Pantel G, Bissery M-C, Brealey C, Lavayre J, Lelièvre Y, Riou J-F, Vrignaud P, Duchesne M, Lavelle F: **I. Target for Cancer and Cardiovascular Therapy.** *In: Farnesyl Transferase and Geranylgeranyl Transferase* 1999
78. Demarcq C, Bastian G, Remvikos Y: **BrdUrd/DNA flow cytometry analysis demonstrates cis-diaminedichloroplatinium (II)-induced multiple cell cycle modifications on human lung carcinoma cells.** *Cytometry* 1992, **13:**416-422
79. Mies B, Rottner K, Small JV: **Multiple immunofluorescence microscopy of the cytoskeleton.** *In: Cell biology: a laboratory handbook* 1998, **2:**469-476
80. Rockwell S: **Effects of clumps and clusters on survival measurements with clonogenic assays.** *Cancer Res* 1985, **45:**1601-1607
81. Hall A: **Ras and GAP – Who's controlling whom?** *Cell* 1990, **61:**921-923
82. Little JB, Hahn GM, Frindel E, Tubiana M: **Repair of potentially lethal radiation damage *in vitro* and *in vivo*.** *Radiology* 1973, **106:**689-694