The New Sulindac Derivative IND 12 Reverses Ras-induced Cell Transformation

Ioanna-Maria Karaguni, Peter Herter, Philip Debruyne, Slava Chatarbova, Alice Kasprzynski, Ulrike Herbrand, M-Reza Ahmadian, Karl-Heinz Glüsenkamp, Günther Winde, Marc Marcel, Tarik Möröy, and Oliver Müller

Max-Planck-Institut für molekulare Physiologie, 44227 Dortmund, Germany [I-M.K., P.H., S.C., A.K., U.H., M.R.A., O.M.]; Laboratory of Experimental Cancerology, Ghent University Hospital, B-9000 Ghent, Belgium [P.D., M.M.]; Squarix GmbH, 45768 Marl, Germany [K.-H.G.]; Klinikum Kreis Herford, Herford, 32045 Germany [G.W.]; and Institut für Zellbiologie (Tumorforschung), Universitätsklinikum Essen, 45122 Essen, Germany [T.M.]

ABSTRACT

The nonsteroidal anti-inflammatory drug Sulindac has chemopreventive and antitumorigenic properties. Its metabolites induce apoptosis and inhibit signaling pathways critical for malignant transformation, including the Ras pathway. Here we show that the new Sulindac derivative IND 12 reverses the phenotype of Ras-transformed MDCK-f3 cells and restores an untransformed epitheloid morphology characterized by growth in monolayers with regular cell-cell adhesions. Moreover, IND 12 treatment induces the expression at membranes of the cell adhesion protein E-cadherin and increases the level of the E-cadherin-bound β-catenin. As a consequence, IND 12-treated MDCK-D cells lose their invasion capacity and regain the ability to aggregate. In the presence of IND 12, MDCK-f3 cells show regenerated expression and activity ratios of the small GTPases Rac and Rho normally found in untransformed MDCK cells. Strikingly, IND 12 treatment decreases the levels of phosphorylated mitogen-activated protein kinases, which are downstream substrates of the Ras-regulated Raf/mitogen-activated protein kinase pathway, and the level of Ras-induced activation of gene expression. Our findings identify a novel drug with high potential in cancer therapy by targeting Ras-induced cell transformation.

INTRODUCTION

Sulindac and other nonsteroidal anti-inflammatory drugs reduce the overall risk to develop cancer and can inhibit the growth of tumors (1–3). Nonsteroidal anti-inflammatory drugs inhibit the key enzymes of the eicosanoid metabolism, the COX 1 and 2, and thereby decrease the levels of proliferation-activating eicosanoids (4). The COX inhibitory effect of Sulindac is mediated by its main physiological metabolite Sulindac sulfide, whereas Sulindac itself and its sulfone metabolite do not affect the COX pathway (5). Nevertheless, Sulindac sulfone, which is ineffective on COX, can induce apoptosis and is able to inhibit tumor growth (6). Additional findings that Sulindac metabolites cause apoptosis in epithelial tissue and in cultured cancer cells independently from the eicosanoid metabolism indicated that Sulindac might exert its antitumorigenic effects independently from the eicosanoid pathway (7–9). Sulindac is used in the therapy and the prevention of tumors in patients with the cancer predisposition familial adenomatous polyposis caused by inherited mutations in the APC gene (7, 10). Experiments performed with the Min mouse, the animal model for familial adenomatous polyposis, also demonstrated that the antitumor effect of Sulindac is independent of prostaglandin biosynthesis (11).

It has been shown that Sulindac metabolites can inhibit Ras-induced signal transduction. The consequences of this inhibition are reduced activation levels of MAP/ERK kinase 1/2, p44-MAPK, and p42-MAPK and decreased levels of Ras-activated gene expression (12–14). Along this line, oncogenic Ras interferes with the induction of cell death by Sulindac metabolites (15, 16). In vitro, Sulindac sulfide directly blocks the interaction between p21ras and its main effector Raf kinase (12). These results offer an explanation for the finding that Sulindac inhibits the growth of tumors harboring Ras gene mutations more effectively than the growth of tumors with the wild-type Ras gene (17).

Recently, we identified a novel Sulindac derivative, named IND 12, which inhibited the proliferation of Ras-transformed cells at significantly lower concentrations than were needed to inhibit the proliferation of untransformed cells (18). In addition, IND 12 inhibited COX activity and the p21ras/Raf kinase interaction at lower concentrations than Sulindac sulfide. On the basis of these results, IND 12 represented a promising candidate in the search for a new antiproliferative drug with inhibitory activity on the Ras pathway. In this study, we asked whether IND 12 can interfere with cell transformation induced by the oncogenic Ras gene. As a model system, we used MDCK cells, which undergo epithelial-to-mesenchymal transition on transformation with oncogenic Ras. Cells of the Ras-transformed subclone MDCK-f3 can be distinguished from the untransformed MDCK parent cell line by several morphological and biochemical parameters (cell morphology, cell growth, cell aggregation, E-cadherin/β-catenin interaction; Ref. 19).

MATERIALS AND METHODS

Overall Experimental Strategy. On the first level of analysis, we screened for drug-induced alterations of cell morphology, cell growth, cell aggregation, and cell invasion behavior. We visualized these parameters by microscopy in cell biological assays. On the next level, we asked for the molecular background of the detected drug-induced effects. To address this question, we analyzed the levels and the activities of proteins, which are known to play a pivotal role in the regulation of cell adhesion and cytoskeletal arrangement. At this point, our results indicated that the drug directly inhibits the Ras-signaling pathway. Therefore, we looked for the activity of the Ras pathway under drug influence by measuring the levels of Ras-activated effector proteins and Ras-induced gene expression.

The Drug IND 12 and the Cell Lines. The synthesis and the chemical properties of IND 12 have been described (18). The drug was dissolved in DMSO as a 1000 × stock solution. In all experiments, DMSO controls showed no significant effects. The linear correlation between drug concentration and UV absorption at the wavelength of the characteristic absorption maximum revealed no hints of insolubility or micelle formation in buffered aqueous solutions at the applied concentrations. Epithelial MDCK cells and MDCK-f3 cells were kindly provided by John G. Collard (Amsterdam). The MDCK-f3 cell line is a subclone of the MDCK-ras-f cell line, which is a virally Ras-transformed derivative of the MDCK cell line (20). MDCK-f3 cells are cytokeratin positive and show a fibroblast-like morphotype (19). Rabbit kidney epithelial-like RK13 cells were purchased from American Type Culture Collection. Cells were cultured under standard conditions.

Fluorescence Microscopy and SEM. The cellular morphology was analyzed by light microscopic or confocal microscopic analysis of cells immuno-

Received 6/15/01; accepted 1/16/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by grants from the Deutsche Forschungsgemeinschaft (Grant MU 1091/8-1) and the Deutsche Krebsshilfe (Grant 10-1474-Mü2). Philip Debruyne is a research assistant with the “Bijzonder Onderzoeksfonds Universiteit Gent.”

2 To whom requests for reprints should be addressed, at Max-Planck-Institut für molekulare Physiologie, Otto-Hahn-Straße 11, 44227 Dortmund, Germany. Phone: +49-231-1332158, Fax: +49-231-1332199; E-mail: oliver.muellar@mpi-dortmund.mpg.de.

The abbreviations used are: COX, cyclooxygenase; MAP, mitogen-activated protein; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MDCK, Madin-Darby Canine Kidney; IND 12, (Z)-5-Fluoro-2-methyl-1-(2-furanyl)-inden-3-acetic acid; SEM, scanning electron microscopy; MAPKK, mitogen-activated protein kinase kinase; F-actin, filamentous-actin.
labeled with a monoclonal mouse rhodamine-coupled antibody directed against F-actin or polyclonal rat antibodies directed against E-cadherin (Sigma Chemical Co.). Binding sites of the primary rat antibodies were detected using a secondary antitrinitro-deriazinylamininemofluorescein-coupled antibody (Transduction Laboratories). For SEM analysis, cells were grown on thermoxed slides and fixed by incubation in 4% paraformaldehyde and 1% glutaraldehyde in 200 mM HEPES (pH 7.4) for 45 min. Fixed cells were dehydrated in an ascending ethanol series and then critical point dried using methanol as intermediate and CO2 as drying medium. To avoid charging artifacts, the dried cells were covered with gold by sputter coating. Covered cells were studied with a Hitachi S-800 SEM equipped with a field emission gun. Cell surface morphology was imaged by the signal of secondary electrons at an accelerating voltage of 20 kV and a working distance of 10 mm.

**Analysis of the E-cadherin/β-catenin Complex by Immunoprecipitation and Western Blot.** Cells were washed with PBS and lysed in cold PBS containing 1% Triton X 100, 1% NP40, and protease inhibitors. Cell debris was precipitated by centrifugation, and supernatants were adjusted to equal concentrations of total protein. Aliquots of the supernatants were retained for the analysis of the total amount of β-catenin. The E-cadherin/β-catenin complex was immunoprecipitated from the supernatants using antibodies against E-cadherin (Transduction Laboratories). The precipitates were analyzed by Western blot probed with a primary monoclonal antibody against β-catenin, or against E-cadherin as a control (Transduction Laboratories), and a secondary rabbit antimouse antibody coupled to horseradish peroxidase (Amersham Pharmacia Biotech). Signals were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech).

**Cell Aggregation Assay and Collagen Invasion Assay.** Slow cell aggregation assay was performed as described (21, 22). Briefly, 20,000 cells/ml were incubated with or without 100 μM IND 12 after seeding on soft agar in wells of a 96-well plate. After 24 h, aggregation was evaluated under an inverted microscope. Collagen invasion assay was performed as described (23, 24). Briefly, 10^3 cells/ml were seeded with or without 100 μM IND 12 on top of collagen type I gels (from rat tail; Upstate Biotechnology), which were prepared in DMEM (0.22% w/v) in six-well plates. After 24 h, cell invasion was evaluated under an inverted microscope. Cells on or above the surface of the collagen gel were regarded as superficial cells. Single individual cells found 10 μm or deeper inside the collagen gel matrix were interpreted as invasive cells that have migrated into the collagen gel. The number of superficial and invasive cells was counted in 15 microscope fields. The invasion index was calculated as a percentage of invasive cells referred to the total number of superficial and invasive cells.

**Rac and Rho Activity Assay.** Sustained activation of the Ras pathway leads to the decrease of the relative level of active GTP-bound Rac what in turn increases the level of GTP-bound Rho (25). The balance of the levels of active Rac and Rho is crucial for cellular morphology (26). The levels of the active small GTapases Rac and Rho were measured as described (25, 26). GTP-bound active Rac or Rho molecules were precipitated by p21-activated kinase or C21, each fused to glutathione-S transferase precoupled to glutathione-Sepharose beads, respectively. The total amount of the GTapases in the cell lysate and the precipitated amount of the GTapases were detected by Western blot analysis using monoclonal antibodies against Rac1 (Transduction Laboratories) or RhoA (Santa Cruz Biotechnology).

**MAP Kinase Assay.** The total levels and the levels of the phosphorylated active kinases p44-MAP and p42-MAP (p44-MAPK/p42-MAPK, also called MAPK1/2 or ERK1/2) were analyzed by Western blot of cell lysates as described (25, 27). The phosphorylated p44-MAP and p42-MAPK proteins were detected by antibodies (Cell Signaling), which bind to the proteins only when activated catalytically by phosphorylation. The total levels of p44-MAPK and p42-MAP were analyzed on a parallel Western blot and detected by antibodies, which bind to p44-MAPK and p42-MAPK independently of phosphorylation (New England Biolabs).

**Reporter Gene Assay.** The reporter gene assay was performed as described (12). RK13 cells were transfected by the calcium phosphate coprecipitation method using the vectors, which have been described (28–30). As controls, the reporter gene was stimulated by transfection with the constitutively active forms of Raf and Raf-CAAX and of MAP-KK and MAP-KK(EE), respectively (12). The MAP-KK(EE) transfection vector was kindly provided by Chris Marshall (London). To test a potential inhibitory effect of the drug on the expression of the reporter gene already from the beginning of the induction, we added 100 μM IND 12 to the medium right after transfection. After transfection (36 h), cells were lysed, and luciferase and β-galactosidase activities were determined. Relative luciferase activity was calculated by normalizing luminescence to β-galactosidase activity.

**RESULTS**

**IND 12 Restores Epithelial Morphology in Malignantly Transformed MDCK-f3 Cells, Reactivates Cell Aggregation, and Inhibits Cell Invasion.** MDCK cells display the typical cellular and cell surface morphology of polarized transporting epithelial cells as revealed by scanning electron and light microscopy (Figs. 2 and 3). MDCK cells are characterized by numerous cell surface microvilli and well-organized cell-cell contacts and grow in an epithelium-like monolayer. In contrast, oncogenically transformed MDCK-f3 cells show the spindle-shaped form of functionally dedifferentiated tumor cells only sparsely covered with microvilli, strong membrane ruffling, and a reduced number of cell-cell contacts. They grow in single cell clones and partly overlap each other indicative for the loss of contact inhibition (Figs. 1–3). To test the effective concentration and the time-dependent effects of IND 12 on MDCK-f3 cells, we monitored morphological changes after IND 12 incubation. We observed that the morphology of MDCK-f3 cells changed in an IND 12 concentration-dependent manner toward the epithelioid phenotype that is characteristic for untransformed MDCK cells (Fig. 1). The maximum effect was reached at 100 μM IND 12. Further increase of the IND 12 concentration showed no additional effect on cellular morphology. Therefore, we chose 100 μM as incubation concentration in the additional assays. IND 12-treated MDCK-f3 cells also resembled untransformed MDCK cells in their growing manner and number of cell contacts (Fig. 2). The treatment of untransformed MDCK cells had no effect. The morphological reversion of MDCK-f3 cells from the fibroblastoid to the epithelioid phenotype was confirmed by electron microscopy to be dependent on the drug-incubation time (Fig. 3). The fibroblastoid MDCK-f3 cells changed to cells with smooth, flattened cell surface with less filopodia compared with untreated MDCK-f3 cells. The extent of this reversion increased time dependently up to an incubation time of 8 h. Longer incubation had no additional effects. Even after 24 h, the IND 12-induced reversion of the transformed into the untransformed phenotype was not complete, e.g., IND 12-treated MDCK-f3 cells did not show as many microvilli as MDCK cells (Fig. 3).

In contrast to MDCK-f3 cells, MDCK cells form cell aggregates when seeded on soft agar (Fig. 4). When MDCK-f3 cells were grown in the presence of IND 12, they regained this ability and formed cell aggregates typical for untransformed MDCK cells. Although the aggregation phenotype of IND 12-treated MDCK-f3 cells is somewhat different from MDCK cells, our results show that IND 12 treatment restores the general ability of MDCK-f3 cells for aggregation and that IND 12 initiates the reversal to the aggregation phenotype of nontransformed cells. Moreover, IND 12 also inhibited the invasion of MDCK-f3 cells into a collagen matrix. The invasion index of IND 12-treated MDCK-f3 cells was decreased by >60% compared with untreated cells (Fig. 5). These results demonstrate that IND 12 is able to restore typical features of an epithelioid phenotype in Ras-transformed cells as contact inhibition, aggregation ability, and the absence of invasive behavior. This was also confirmed by the observation that IND 12-treated MDCK-f3 on the top of the collagen matrix showed very similar phenotype as MDCK cells (data not shown).

**IND 12-treated MDCK-f3 Cells Regain the Levels of the E-cadherin/β-catenin Complex and the Relative Levels of Active Rac and Rho.** Whereas the cell adhesion membrane protein E-cadherin is barely detectable in the Ras-transformed MDCK-f3 cells,
the treatment of MDCK-f3 cells with IND 12 led to the increase of the level of membrane-bound E-cadherin (Fig. 2). IND 12 treatment also led to the re-expression of the E-cadherin/β-catenin complex to levels normally found in untransformed MDCK cells (Fig. 6). In contrast, the level of total β-catenin remained unchanged in MDCK-f3 cells after IND 12 treatment, indicating that the observed increase of the E-cadherin/β-catenin complex is because of an increase in the level of membrane-bound E-cadherin that recruits cytosolic β-catenin (Figs. 2 and 6).

To gain further insight into the molecular mechanisms that are responsible for the observed morphological alterations, we analyzed the levels of the active GTPases Rac and Rho.
treatment led to a significant increase of the amount of active Rac relative to the amount of total Rac (Fig. 7). Conversely, the level of active Rho was lowered in the presence of IND 12 reaching the intrinsic level of active Rho in untransformed cells after 8 h. These results suggest that the morphological shift of transformed MDCK-f3 cells observed on IND 12 treatment is associated with a restoration of the Rac/Rho ratio normally found in untransformed MDCK cells.

**IND 12 Inhibits the Ras Pathway.** Finally, we tested whether IND 12 had a direct influence on Ras-mediated signal transduction and analyzed the levels of phosphorylated p44-MAPK and p42-MAPK, which are both substrates of the kinases MAP/ERK kinase 1/2, which themselves are activated by the main Ras effector kinase Raf-1. IND 12 treatment reduced the amount of phosphorylated, active p44-MAPK and p42-MAPK (Fig. 8). To measure the effect of IND 12 on the signaling activity of the Ras pathway, we performed a reporter gene assay. In this assay, the Ras-induced transactivation is analyzed (28). IND 12 did not affect the basal activity of the reporter activity (Fig. 9). However, in the presence of IND 12, the luciferase reporter gene activity induced by the oncogenic mutant gene RasG12V was decreased in dependence of the drug concentration compared with the control in the absence of the drug (Fig. 9). In the presence of 100 µM IND 12, the reporter gene activity was decreased by ~70%. Next, we cotransfected the gene for the p21ras effector Raf kinase in a constitutively activated form, Raf-CAAX, to test whether IND 12 acts up or downstream of Raf. The inhibition of transactivation induced by Raf-CAAX was ~45%, indicating an effect of this drug downstream of p21ras. Secondly, the Raf kinase substrate MAP-KK in its constitutively activated form, MAP-KK(EE), was cotransfected. The drug had no effect on the reporter gene activated by MAP-KK(EE), indicating an effect upstream of MAP-KK. In this assay, we chose an incubation time of 36 h to ensure that the drug affects the Ras-induced transactivation of the reporter gene expression from the time of transfection. Although a correlation of the reporter gene assay data with the results, which were received after shorter incubation time, is critical, these data indicate an inhibition of the Ras-activated gene expression by IND 12.

**DISCUSSION**

In a recent study, we found that the sulfide metabolite of Sulindac inhibits the Ras pathway at micromolar concentrations (12). To identify new drugs that can inhibit the Ras pathway more efficiently and at lower concentrations, we synthesized new compounds based on Sulindac as a lead structure (18). One of these, IND 12, showed antiproliferative effects on cultured tumor cells and a lower unspecific...
A SULINDAC DERIVATIVE WHICH REVERSES RAS TRANSFORMATION

As a first sign for the reversion of transformation, IND 12 changed the fibroblastoid phenotype of MDCK-f3 cells toward the epithelioid morphology of the untransformed, normal MDCK cells. Cell-cell contacts are formed by membrane proteins of the cadherin superfamilies, which are the starting point of the molecular cascade that leads to the formation of junctional desmosomes and tight junctions (31). On the cytoplasmic side, cadherins bind to the catenins, which is a prerequisite for the link between cell-cell contacts and the cytoskeleton and, thus, for the stability of cell-cell adhesion. Ras-transformed MDCK-f3 cells are highly invasive as a result of reduced E-cadherin-mediated cell-cell adhesion (19, 20). A reversion of MDCK cell transformation is accompanied by the translocation of cytosolic β-catenin to the membrane and the increase of E-cadherin/β-catenin complex (26). The reversal of the transformed phenotype by IND 12 correlated with a partial restoration of the levels of membrane-bound E-cadherin and of E-cadherin-bound β-catenin. This suggests that the observed morphological changes induced by IND 12 in these cells are based on a reorganization of both molecules at the membrane or in complexes, respectively. These results also show that IND 12 can initiate restoration of membrane-bound E-cadherin, retranslocation of β-catenin to the cell membrane, and the reformation of complexes between these molecules. Thus, it is conceivable that IND 12-treated cells regained the ability for contact inhibition and lose their invasive potential. The exact molecular mechanism by which IND 12 can exert this reversal of phenotype and the restoration of the levels of the membrane-bound E-cadherin and of the E-cadherin/β-catenin complex remains to be determined. However, the morphological differences between MDCK-f3 and MDCK cells have been shown to be a consequence of Ras-induced transformation of MDCK-f3 cells (19, 20). In this model, sustained Ras activation leads to the down-regulation of the relative level of active Rac in MDCK-f3 cells (25). Rac plays a role as a suppressor of invasion of epithelial cells and regulates cytoskeletal rearrangement and epithelial morphology and, thus, plays a role in the control of cell morphology and migration (26, 32). As a result of down-regulated Rac activity, the level of the active GTPase Rho increases. We show here that in the presence of IND 12, the ratio of active Rac and Rho levels relative to the total levels of the two proteins is altered in MDCK-f3 cells and resembles the ratios normally found in untransformed MDCK cells. These findings sug-

cytotoxicity than Sulindac. In a proliferation assay, IND 12 inhibited the growth of MDCK-f3 cells that had been transformed by oncogenic Ras at a six times lower concentration than the growth of MDCK control cells (18). Furthermore, IND 12 could interfere with the interaction of the p21ras protein with the effector kinase Raf in vitro. Therefore, IND 12 was selected as a candidate compound with high potential in the inhibition of the oncogenically activated Ras pathway and, thus, in the therapy of Ras-induced malignancies. The ability of IND 12 to reverse the transformed phenotype and, thus, the epithelioid-to-mesenchymal transition of transformed MDCK-f3 cells, which is shown in this study, underscores the potential of IND 12 as an anticancer drug.

Fig. 7. The reciprocal levels of active Rac and Rho in MDCK-f3 cells are reconstituted partly by IND 12 to levels found in untreated MDCK cells. A, Western blot analysis showing the p21-activated kinase-complexed active Rac (top row) and the level of total Rac (bottom row) from MDCK, MDCK-f3, and MDCK-f3 cells after treatment with 100 μM IND 12 for the indicated time. In MDCK-f3 cells, the level of active Rac relative to the amount of total Rac was lower than in untreated MDCK cells. IND 12 treatment of MDCK-f3 cells induced the increase of the level of active Rac relative to its total amount after 4 and 8 h. The level of total Rac remained unchanged during IND 12 treatment. Although, the relative level of active Rac to total Rac did not reach the relative level of active Rac in untreated MDCK cells. B, Western blot analysis showing the C21-complexed active Rho (top row) and the level of total Rho (bottom row) from MDCK, MDCK-f3, and MDCK-f3 cells after treatment with 100 μM IND 12 for the indicated time. The level of active Rho was decreased in IND 12-treated MDCK-f3 cells reaching the level of active Rho in untransformed MDCK cells after 8 h, whereas the level of total Rho remained unchanged.

Fig. 8. IND 12 reduces the levels of the active MAP kinases. The levels of the phosphorylated active p44-MAPK and p42-MAPK (top row) and of the total levels of p44-MAPK and p42-MAPK (bottom row) were analyzed by Western blot of protein extracts from MDCK, MDCK-f3, and MDCK-f3 cells treated with 100 μM IND 12 for the indicated time using specific antibodies. As expected, the levels of active phospho-p44-MAPK and phospho-p42-MAPK were increased in Ras-transformed MDCK-f3 cells. Treatment of MDCK-f3 cells with IND 12 led to the decrease of the levels of the active MAP kinases (top row), whereas the total level of MAP kinases remained unchanged (bottom row).

Fig. 9. IND 12 inhibits Ras-dependent transactivation. Relative activities of the luciferase reporter gene are shown. The influence of IND 12 was measured on RasG12V-, Raf-CAAX-, or MAP-KK(EE)-induced reporter gene activity, respectively. The activities of the untreated controls were set as 100% of the relative reporter activity. IND 12 inhibits the transcriptional transactivation induced by activated RasG12V in a concentration-dependent manner. IND 12 inhibits also transcriptional transactivation stimulated by Raf-CAAX, whereas it has no effect on the reporter gene activity activated by MAP-KK(EE). Each experiment was performed in triplicates, and average values are given.
gest that the observed effects of IND 12 on the transformed MDCK-f3 cells are associated with the expression or activity of these small G-proteins. Because the alteration of the levels of active Rac and Rho in MDCK-f3 cells is because of the sustained activation of the Ras pathway, an inhibition of the Ras pathway by IND 12 would offer an attractive explanation for the restoration of the Rac and Rho levels and the observed effects of IND 12 on MDCK-f3 cells. A number of other findings suggest that IND 12 exerts its effect by directly interfering with Ras-initiated signal transduction: (a) the levels of the phosphorylated active downstream effectors p44-MAPK and p42-MAPK decreased in the presence of IND 12; (b) IND 12 lowered the gene expression activated by oncogenic Ras but not the gene expression activated by the Ras downstream effector MAP-kinase (EE); and (c) in a recent study, we showed that IND 12 inhibits the interaction between the proto-oncoprotein p21ras and the Raf kinase in vitro (18). Altogether, our findings make IND 12 a promising candidate with high potential in the all-important search for new antitumorigenic drugs with effects on the Ras pathway. Future studies have to demonstrate the effects of this drug on tumors in vivo.

ACKNOWLEDGMENTS

We thank Alfred Wittinghofer for continuous support.

REFERENCES

The New Sulindac Derivative IND 12 Reverses Ras-induced Cell Transformation

Ioanna-Maria Karaguni, Peter Herter, Philip Debruyne, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/6/1718

Cited articles
This article cites 29 articles, 17 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/6/1718.full.html#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
/content/62/6/1718.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.