Tyrosine kinase of insulin-like growth factor receptor as target for novel treatment and prevention strategies of colorectal cancer

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Abstract
AIM: To investigate the antineoplastic potency of the novel insulin-like growth factor 1 receptor (IGF-1R) tyrosine kinase inhibitor (TKI) NVP-AEW541 in cell lines and primary cell cultures of human colorectal cancer (CRC).

METHODS: Cells of primary colorectal carcinomas were from 8 patients. Immunostaining and crystal violet staining were used for analysis of growth factor receptor protein expression and detection of cell number changes, respectively. Cytotoxicity was determined by measuring the release of the cytoplasmic enzyme lactate dehydrogenase (LDH). The proportion of apoptotic cells was determined by quantifying the percentage of sub-G1 (hypodiploid) cells. Cell cycle status reflected by the DNA content of the nuclei was detected by flow cytometry.

RESULTS: NVP-AEW541 dose-dependently inhibited the proliferation of colorectal carcinoma cell lines and primary cell cultures by inducing apoptosis and cell cycle arrest. Apoptosis was characterized by caspase-3 activation and nuclear degradation. Cell cycle was arrested at the G1/S checkpoint. The NVP-AEW541-mediated cell cycle-related signaling involved the inactivation of Akt and extracellular signal-regulated kinase (ERK) 1/2, the upregulation of the cyclin-dependent kinase inhibitors p21[\text{WAF1/CIP1}] and p27[Kip1], and the downregulation of the cell cycle promoter cyclin D1. Moreover, BAX was upregulated during NVP-AEW541-induced apoptosis, whereas Bcl-2 was downregulated. Measurement of LDH release showed that the antineoplastic effect of NVP-AEW541 was not due to general cytotoxicity of the compound. However, augmented antineoplastic effects were observed in combination treatments of NVP-AEW541 with either 5-FU, or the EGFR-antibody cetuximab, or the HMG-CoA-reductase inhibitor fluvastatin.

CONCLUSION: IGF-1R-TK inhibition is a promising novel approach for either mono- or combination treatment strategies of colorectal carcinoma and even for CRC chemoprevention.

Key words: Insulin-like growth factor receptor; Tyrosine kinase; Colorectal cancer; Apoptosis; Cell cycle arrest

INTRODUCTION
Colorectal cancer (CRC) is the third most common cancer and the fourth most frequent cause of cancer-related deaths worldwide. Long-term survival of colorectal cancer is related to the stage of disease. Once distal metastases develop the prognosis is poor[2]. At least 40% of patients with colorectal cancer develop distal metastases and most of them die thereof[3]. Hence, innovative approaches are urgently needed to improve the treatment of advanced colorectal cancer.

There is evidence that insulin-like growth factor 1 receptor (IGF-1R) may be a promising protein for specific and targeted therapeutic approaches[4,5]. Several reports indicate that IGF-1R is overexpressed in the majority (> 90%) of colorectal carcinomas, most likely contributing to the aggressive growth characteristics of these tumors and the poor prognosis[6,7]. IGF-1R is a tetrameric transmembrane receptor tyrosine kinase (TK) composed of two \(\alpha\) and two \(\beta\) subunits. The extracellular \(\alpha\) subunit is responsible for ligand binding, whereas the \(\beta\) subunit consists of a transmembrane domain and a cytoplasmic tyrosine kinase domain[8]. The receptor is predominantly activated by IGF-1 and II but can also be activated by insulin at a much lower affinity (500-1000 fold less). Ligand binding activates in-
trinic tyrosine kinase activity, resulting in trans-β subunit autophosphorylation and stimulation of signaling cascades that include IRS-1/PI-3K/PKB/S6K and Grb2/Sos/Ras/MAPK pathways.[8-10]

In general, both IGFs and IGF-1R are involved in the development and progression of several cancers.[8-10] Activation of IGF-1R by its ligands results in proliferation, survival, transformation, metastasis, and angiogenesis. Hence, abnormal or enhanced expression of IGFs and IGF-1R has been correlated with disease stage, reduced survival, metastasis development and de-differentiation of a broad variety of tumors. Obesity and diabetes are associated with an increased risk of colorectal cancer.[11] This effect seems to be due to alterations in the metabolism of endogenous hormones, including sex steroids, insulin and also activation of the IGF/IGF-receptor system which further supports the idea of the IGF/IGF-receptor system to be a promising target for colorectal cancer treatment and chemoprevention. Several studies have demonstrated the therapeutic potential of interfering with IGF-1R-mediated signaling in cancer cells in vitro and in vivo. These approaches include the use of antagonistic IGF-1R antibodies, IGF-1R antisense oligonucleotides, or IGF-1R siRNA.[12-14]. Specific inhibition of IGF-1R TK activity appears to be another promising principle.

Recently, NVP-AEW541, an orally available low-molecular-weight pyrrolo [2, 3-d] pyrimidine derivative, has been introduced as a potent and reversible inhibitor of IGF-1R tyrosine kinase activity.[15] NVP-AEW541 has been shown to be highly selective for IGF-1R-TK, as compared to both the closely related insulin receptor (InsR) and other tyrosine or serine/threonine kinases. Antitumor activity of NVP-AEW541 has already been demonstrated in fibrosarcomas and breast cancer.[16] IGF-R TK inhibition has not been evaluated for the treatment of colorectal cancer. Hence, in the present study we examined the antineoplastic potency of NVP-AEW541 both in human colorectal carcinoma cell lines and in primary cell cultures of human CRC, which shows that NVP-AEW541 potently inhibits colorectal cancer growth by inducing apoptosis and cell cycle arrest in human colorectal carcinoma cells.

MATERIALS AND METHODS

Cell lines and drugs

The human colorectal adenocarcinoma cell line HT29 was cultured in RPMI 1640 medium supplemented with 100 g/L fetal bovine serum, penicillin (100 kU/L), and streptomycin (100 mg/L). The human colorectal adenocarcinoma cell line HCT116 was grown in Dulbecco’s minimal essential medium containing 100 g/L fetal bovine serum, penicillin (100 kU/L), and streptomycin (100 mg/L). Cells were kept in a humidified atmosphere (50 mL/L CO2) at 37°C. Cells were incubated with culture medium containing NVP-AEW541 (Novartis, Basel, Switzerland). For combination treatment, cells were incubated simultaneously with NVP-AEW541 and one of the following drugs: 5-fluorouracil (Sigma, Deisenhofen, Germany), SN-38 (Rhone-Poulenc Rorer, Antony, France), cetuximab (Merck KgaA, Darmstadt, Germany) or fluvastatin (Calbiochem-Novabiochem, Bad Soden, Germany). Stock solutions (in DMSO, stored at -20°C) were diluted to the final concentration in fresh media before each experiment. In all experiments, the final DMSO concentration did not exceed 1 mL/L

Isolation and establishment of primary cell cultures from human colorectal cancers

Cells of primary colorectal carcinomas from 8 patients (5 males, 3 females, age range 74 ± 14 years) were isolated from endoscopically taken biopsies as previously described.[17] The human tumor material was used according to the standards set by the Ethical Committee of the Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany. Cell preparation was performed by incubation (30 min/RT) with a solution containing 0.5 g/L trypsin, 0.2 g/L EDTA and 1 g/L collagenase. The isolated human colorectal carcinoma cells were maintained in Earle’s 199 medium supplemented with 200 g/L FBS, 2 mmol/L L-glutamine, 20 g/L Biotect protective medium, penicillin (100 kU/L), streptomycin (100 mg/L), 10 g/L amphotericin B, and incubated at 37°C in a humidified atmosphere (50 mL/L CO2).

Analysis of growth factor receptor expression

For analysis of growth factor receptor protein expression, cells were immunostained as previously described.[18,19] In brief, samples were fixed, permeabilized, and subsequently incubated with a polyclonal anti-EGFR or IGF-1R antibody (5 mg/L, BD Pharmingen, Heidelberg, Germany). Cell preparation was performed by flow cytometry on a FACSCalibur (Becton Dickinson, Heidelberg, Germany) and analyzed using CellQuest software.

Determination of cell number

Cell number was evaluated by crystal violet staining as previously described.[20] In brief, cells in 96-well plates were fixed with 10 mL/L glutaraldehyde and then stained with 1 g/L crystal violet. The unbound dye was removed by washing with water. Bound crystal violet was solubilized with 2 g/L Triton X-100. Light extinction increasing linearly with the cell number was analyzed at 570 nm using an ELISA reader.

Determination of cytotoxicity

Cells were incubated with 0-10 μmol/L NVP-AEW541 for 1, 6, 12 and 24 h. Release of the cytoplasmic enzyme lactate dehydrogenase (LDH), indicating cytotoxicity, was measured using a colorimetric kit from Roche (Roche Diagnostics, Mannheim, Germany) as described previously.[21,22] Background release from untreated cells was subtracted. Maximum release was measured after adding 2 g/L Triton X-100 to untreated cells. For determinations, LDH assay reagent was added to sample supernatants and incubated for 30 min at room temperature in dark. Absorbance was measured at 490 nm (reference wavelength 690 nm).

5636  ISSN 1007-9327  CN 14-1219/R  World J Gastroenterol  September 21, 2006  Volume 12  Number 35

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Detection of apoptosis
Preparation of cell lysates and determination of caspase-3 activity were performed as previously described\textsuperscript{[23]}. The activity of caspase-3 was calculated from the cleavage of the fluorogenic substrate DEVD-AMC (Calbiochem-Novabiochem, Bad Soden, Germany). The proportion of apoptotic cells was determined by quantifying the percentage of sub-G1 (hypodiploid) cells after flow cytometric analysis of isolated propidium iodide-stained nuclei\textsuperscript{[24].

Cell cycle analysis
Cell cycle analysis was performed by the method of Vindelov and Christensen\textsuperscript{[25]. Cells were trypsinized, washed, and the nuclei were isolated using CycleTest PLUS DNA Reagent Kit (Becton Dickinson). DNA was stained with propidium iodide according to the manufacturer’s instructions. DNA content of the nuclei was detected by flow cytometry and analyzed using CellFit software (Becton Dickinson).

Western blotting
Western blotting was performed as previously described\textsuperscript{[23]}. Blots were blocked in 50 g/L non-fat dry milk for 1 h, and then incubated at 4°C overnight with anti-phospho-Akt, Akt (both 1:1000, Cell Signaling, Beverly, MA), Bax (1:1000, Santa Cruz Biotechnology, CA), Bel-2 (1:200, Novo Castra Laboratories, Newcastle upon Tyne, UK), COX-2 (1:200), cyclin D1 (1:100), p21\textsuperscript{野生} (1:200), IGF-1R (1:200), phospho-ERK1/2, ERK1/2 (1:1000, Santa Cruz Biotechnology, CA), or p27\textsuperscript{野生} (1:2500, Becton Dickinson). β-actin (1:5000, Sigma) served as a loading control.

Statistical analysis
If not stated otherwise, means of four independent experiments ± SE were shown. Individual drug treatment was compared by the unpaired, two-tailed Mann-Whitney U-test. P < 0.05 was considered statistically significant.

RESULTS
Expression of IGF-1R in colorectal carcinoma cells
Expression of IGF-1R and epidermal growth factor receptor (EGFR) was investigated in human colorectal carcinomas. Protein expression of IGF-1R was detected in both cell lines. In addition, expression of EGFR protein was detected in both cell lines (Figure 1A). IGF-1R protein expression of NVP-AEW541-treated colorectal cancer cells was determined by Western blotting. HT-29 cells incubated with NVP-AEW541 (0-10 μmol/L) for 48 h did not abolish the expression of IGF-1R. By contrast even after treatment with 10 μmol/L of NVP-AEW541, a robust expression of IGF-1R protein could still be observed (Figure 1B). IGF-1R and EGFR expression was confirmed in the investigated 8 primary colorectal cancer cultures by RT-PCR using established primers (not shown)\textsuperscript{[18,22].

NVP-AEW541-induced growth inhibition of colorectal carcinoma cells
Cell number changes caused by IGF-1R-TK inhibition with NVP-AEW541 were studied by crystal violet assays. NVP-AEW541 time- and dose-dependently inhibited the growth of HT-29 and HCT-116 cells (Figure 2A and B). The IC_{50} values of NVP-AEW541 were 1.7 ± 0.4 μmol/L (HT-29) and 2.5 ± 0.4 μmol/L (HCT-116), as determined after 4 d of incubation.

In line with our findings in permanent cell lines, NVP-AEW541 treatment (0-5 μmol/L) reduced the cell number of primary cultures of human colorectal carcinomas in a dose-dependent manner. After 3 d of incubation a cell number reduction of 47.3% ± 2.4% was detected by direct cell counting in six NVP-AEW541-sensitive primary culture preparations. Two out of the investigated 8 primary cultures displayed only a weak growth inhibition of 12% ± 4%. In treatment-sensitive primary cultures NVP-AEW541 also altered the morphology of the remaining cells, which appeared shrunken and flat. Propidium iodide-positive staining of primary culture cells revealed that NVP-AEW541 treatment led to a loss of cell membrane integrity indicating cell death or that these cells were in the process of dying, respectively (Figure 2C).

Antineoplastic potency of NVP-AEW541 in combination with cytostatics, cetuximab or the HMG-CoA reductase inhibitor fluvastatin
To test whether combination treatment of NVP-AEW541
with either 5-fluorouracil (5-FU), SN-38, or whether the humanized monoclonal anti-EGFR antibody cetuximab may lead to additive antiproliferative effects, HT29 and HCT-116 cells were co-treated for 96 h with sub-IC\textsubscript{50} concentrations of NVP-AEW541 (1 μmol/L) plus either 5-FU (10 μmol/L), or cetuximab (1000 mg/L), or SN-38 (10 nmol/L). In both colorectal carcinoma cell models combination treatment with NVP-AEW541 plus either 5-FU (Figure 3A) or cetuximab (Figure 3B) resulted in additive or even over-additive growth inhibitory effects. When NVP-AEW541 was combined with SN-38, additive antiproliferative effects were only observed in HCT-116 cells (Figure 3C). We additionally investigated the anti-proliferative potency of the HMG-CoAR inhibitor fluvastatin, either alone or in combination with NVP-AEW541 in colorectal carcinoma cells (Figure 4). Fluvastatin (0-50 μmol/L) caused a dose-dependent growth inhibition of more than 80% both in HCT-116 cells and in HT-29 cells. Combinations of sub-IC\textsubscript{50} concentrations of fluvastatin and NVP-AEW541 resulted in an additive growth inhibi-
tion of either colorectal cancer model.

**NVP-AEW541-induced apoptosis in colorectal cancer cells**

NVP-AEW541 dose-dependently induced a significant increase of caspase-3 activity after 24 h of incubation (Figure 5A). Compared to control cells, an increase of up to 500% was observed. The dose-dependent induction of apoptosis by NVP-AEW541 became also apparent by flow cytometrically monitoring nuclear degradation after 72 h of treatment (Figure 5B).

**NVP-AEW541-induced cell cycle arrest in colorectal carcinoma cells.**

To test whether an induction of cell cycle arrest contributed to the antiproliferative potency of NVP-AEW541 in colorectal carcinoma cells, we performed cell cycle analyses. NVP-AEW541 dose-dependently arrested HT-29 and HCT-116 cells in the G1/G0 phase of the cell cycle after 24 h of treatment, thereby decreasing the proportion of cells in the S and G2/M phases (Figure 6).

**Cytotoxicity of NVP-AEW541**

Cytotoxicity of NVP-AEW541 was determined by measuring LDH release. HT29 and HCT-116 cells incubated with 1-10 μmol/L NVP-AEW541 for 1, 6 or 12 h did not result in a measurable increase in LDH release, indicating that NVP-AEW541 did not directly affect cell membrane integrity. After 24 h of incubation a slight but not significant increase in LDH-release of about 3% was observed at 10 μmol/L NVP-AEW541, indicating that even at high concentrations NVP-AEW541 did not cause immediate necrotic/cytotoxic effects in colorectal cancer cells (data not shown).

**NVP-AEW541-induced modulation of cell cycle and apoptosis-related signaling molecules**

The effects of NVP-AEW541 on the phosphorylation of both ERK1/2 and its upstream regulator Akt/PKB were investigated to elucidate the signaling pathways modulated by IGF-1R-TK inhibition. NVP-AEW541 treatment dose-dependently decreased the phosphorylation of both mitogenic and antiapoptotic ERK1/2 MAPK as well as Akt/PKB (Figure 7A). Next, the expression of cell cycle-related proteins was investigated to explore the pathway downstream of NVP-AEW541-induced dephosphorylation of Akt/PKB and ERK1/2, known to be causative for cell cycle arrest. NVP-AEW541 decreased the expression of cyclin D1 but increased the expression of the CDK inhibitors p21<sup></sup>Waf1/CIP1<sup></sup> and p27<sup></sup>Kip1<sup></sup>. These data suggest that NVP-AEW541-induced cell cycle arrest was mediated by p21<sup></sup>Waf1/CIP1<sup></sup> and p27<sup></sup>Kip1<sup></sup> induction, resulting in a decrease of cyclin D1.

To survey the proapoptotic signaling pathways modulated by IGF-1R-TK inhibition with NVP-AEW541 in CRC...
cells, we also investigated the effects of NVP-AEW541 on the expression pattern of Bcl-2 and Bax. Treatment with NVP-AEW541 dose-dependently increased the expression of the proapoptotic Bax protein, while the expression of the antiapoptotic protein Bcl-2 slightly decreased. Finally, it was proved that NVP-AEW541 could downregulate the expression of cyclooxygenase 2 (COX-2) in colorectal cancer cells (Figure 7B).

**DISCUSSION**

Treatment options for advanced colorectal cancer (CRC) are unsatisfactory. Thus, there is a strong need for effective novel treatment strategies of CRC. A novel approach for CRC treatment may be the interruption of IGF/IGF-receptor signaling system, which is known to have strong stimulatory effects on cancer cell growth. The protective and mitogenic effects of the IGF/IGF-receptor system involve the constitutive activation of antiapoptotic proteins as well as cell cycle promoting signaling. A tight association between IGF-receptor signaling and regulation of cell growth and apoptosis in CRCs which commonly overexpress IGF-receptors has been described.[25,26]

Most fast growing cancers produce and release growth factors and thereby auto-stimulate their growth. This also holds true for CRC in which epidermal growth factor (EGF), transforming growth factor α (TGF-α), insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF) are produced and secreted to promote CRC growth.[27]. Therefore, the antiproliferative effects of the novel IGF-1R tyrosine kinase inhibitor NVP-AEW541 were investigated under serum-containing conditions (e.g. in the presence of growth factors like EGF, IGF and TGF-α). Our study demonstrated that inhibition of IGF-1R tyrosine kinase activity by NVP-AEW541 might be suitable for novel targeted therapy of CRC. By abrogating the protective and mitogenic effects of IGF-R signaling, CRC cell growth was potently inhibited by NVP-AEW541. The antineoplastic effects of NVP-AEW541 were based on a pronounced induction of cell cycle arrest and apoptosis. Accompanying or dose-limiting cytotoxicity was not observed, underlining the specific mode of action of the drug. Our findings are in line with recent observations on IGF-R inhibition in other cancer models. Although applying other approaches for IGF-R inhibition, these studies have also shown the strong antineoplastic potency of the interruption of IGF-R signaling without dose-limiting toxicity in several other cancer models.[16,28,29]

Re-initialisation of apoptosis and induction of cell cycle arrest are valuable features for a successful anti-cancer agent. On the one hand, apoptotic cell death is not accompanied with undesired immunological reactions, which occur upon treatment with unspecific and cytotoxic
agents. On the other hand, apoptosis-sensitization and cell cycle arresting effects offer possibilities for powerful combination treatments. In this respect, we investigated combination treatments of NVP-AEW541 and clinically relevant cytostatics with different modes of action. The tested combinations of DNA strand-breaking pyrimidine analog 5-fluorouracil (5-FU) and topoisomerase-I inhibitor SN-38, except for the combination of NVP-AEW541 and SN-38 in HT29 cells, resulted in additive antiproliferative effects. The additive antineoplastic potency appeared to be independent of the mode of action of the respective cytostatic drug. Our data support the notion that NVP-AEW541 is a promising new agent for potentiation of antitumor efficacy of the established cytostatic CRC treatment.

It has been pointed out that the antipotopic potency of IGF/IGFR-signaling might interfere with strategies that target other tyrosine kinases such as EGFR-TK. Hence, the antineoplastic potency of EGFR blockade may well be underestimated when examined under conditions where IGF-1R is fully functional. IGF-1R is capable of transactivating EGFR-TK and abrogating the antiproliferative effects of EGFR-antibody treatments. As the CRC cell models used in this study were shown to express both IGF-1R and EGFR, we studied the effects of targeting both growth factor receptors by combination treatment. Treating CRC cells with sub-IC50 concentrations of both NVP-AEW541 and humanized anti-EGFR-antibody cetuximab enhanced the antiproliferative effect as compared to the effect of either agent alone. Thus, NVP-AEW541 qualifies as a promising substance for combination treatment strategies to overcome the compensatory effects of mitogenic crosstalks between IGF-1R and EGFR in CRC.

Drug resistance is one of the major problems of chemotherapy. Potential mechanisms of drug resistance include the activation of Ras/Raf-Mek/ERK signal transduction cascade and the increase of cholesterol levels in cancer cells, both being controlled by isoprenoids. The production of isoprenoids is catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR) which may therefore be a rational molecular target for innovative antineoplastic treatment of colorectal cancer. Fluvastatin is an effective inhibitor of HMG-CoAR and has already been shown to inhibit tumor cell growth.

In the present study, we have demonstrated the antineoplastic effect of fluvastatin alone and in combination with NVP-AEW541 in colorectal cancer cells and the antiproliferative effect of NVP-AEW541 augmented by fluvastatin, suggesting that combining NVP-AEW541 and fluvastatin may be a promising approach for dual targeting treatment strategies in colorectal cancer disease.

The mechanisms underlying the antiproliferative action of NVP-AEW541 in colorectal cancer cells were further characterized. NVP-AEW541 induced cell cycle arrest in the G1/G0-phase in both CRC cell lines, suggesting that the drug acts at the G1/S checkpoint. A G1/S cell cycle arrest induced by inhibition of IGFR signaling has been described in other fast growing cancers. Moreover, we observed a definite rise in apoptotic cells after treatment with NVP-AEW541. While the induction of apoptosis is a well-known effect occurring upon inhibition of IGFR-signaling, the underlying mechanisms have been poorly characterized. Our present results suggest that activation of caspase-3 is involved in NVP-AEW541-induced apoptosis of CRC cells. The proapoptotic protein BAX was upregulated during NVP-AEW541-induced apoptosis, while Bcl-2, the protective and antiapoptotic counterpart of Bax, was downregulated. Our findings suggest that involvement of mitochondrial pathways leads to NVP-AEW541-mediated apoptosis.

As activated IGF-1R induces the Ras-Raf-MEK-ERK signaling pathway with subsequent induction of cyclin D1 expression, we analyzed NVP-AEW541-induced changes of Akt/PKB, ERK1/2 activity and p21Waf1/Cip1, p27Kip1 as well as cyclin D1 expression as previously described. In agreement with previous observations in non-colorectal tumor models, we found that NVP-AEW541 could depophosphorylate IGF-1R as well as ERK1/2 MAPK and AKT/PKB of colorectal cancer cells.

IGF-1R has at least three survival signals that are able to protect cancer cells from apoptosis, namely PI-33K/AKT and MAPK/ERK signaling pathways, and a third one that results in the mitochondrial translocation of Raf 1, the so-called 3-3 pathway. Simultaneous inactivation of two of these pathways is required to inhibit IGF-1R capacity of protecting cells from apoptotic injuries. Thus, the simultaneous inactivation of AKT/PKB and ERK1/2 MAPK by NVP-AEW541, we hypothesise that blockade of these two survival pathways is directly involved in the successful inhibition of colorectal cancer cell growth by NVP-AEW541.

Defective function of cell cycle regulators is a main cause for tumor development and progression. For example, the cell cycle promoter cyclin D1 is frequently overexpressed in CRC. Successful therapeutic strategies have to balance or bypass the impaired signaling. In the present study, NVP-AEW541 treatment raised the expression of the cell cycle-inhibiting molecules p21Waf1/Cip1 and p27Kip1, while it decreased the expression of cyclin D1. Finally, the expression of COX-2 which is known to be upregulated during colorectal carcinogenesis and plays an important role in colorectal cancer growth, was suppressed by IGF-1R inhibition as previously described. Thus, NVP-AEW541-induced COX-2 suppression may well contribute to its antineoplastic potency in colorectal cancer. Since both COX-2 and IGFR are up-regulated during colorectal carcinogenesis, NVP-AEW541 appears to be a promising chemopreventive agent in patients at risk for CRC.

Primary cell cultures of human colorectal cancers were established as a tool to design a rational individual medical treatment of an individual patient. The primary goal was to study NVP-AEW541’s antineoplastic potency in a bench to bedside approach, as permanent cell lines may represent well-suited but nevertheless non-representative models of colorectal cancers. Moreover, chemosensitivity testing of primary cultures was performed to establish a new method for predicting the response of an individual patient to a certain drug. Attempts to predict individual responses have already been undertaken for breast cancer and colorectal cancer, respectively. Such an approach...
may pave the way to an individualized medical treatment of cancer patients. The importance of predictive testing is further supported by our finding that two out of the tested eight primary cell cultures showed only a weak response to NVP-AEW541 treatment. Nevertheless, NVP-AEW541 is a promising compound for future colorectal cancer treatment, as 75% of the investigated primary colorectal cancers could be effectively treated with the drug.

In conclusion, the IGFR-TK inhibitor NVP-AEW541 potently inhibits the growth of human colorectal cancer cells by inducing both cell cycle arrest and apoptosis without eliciting unspecific cytotoxicity. Furthermore, the compound is well-suited for combination treatment approaches. Thus, inhibition of IGFR-signaling by NVP-AEW541 is a promising targeted anticancer strategy for colorectal carcinoma and should be tested in future clinical trials. Moreover, investigations should be pursued to modulate the IGF/IGFR system as a possible means of chemoprevention of colorectal cancer in patients at risk.

ACKNOWLEDGMENTS

We are indebted to Antje Krahn and Benjamin Becker for expert technical assistance. We thank Novartis for providing us with NVP-AEW541, Dr. Faiss and the nurses of the Central Endoscopy Unit of the Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin for their excellent support of the study. Moreover, we would like to thank the Institute of Physiology, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, for its laboratory facilities.

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S- Editor Wang GP  L- Editor Wang XL  E- Editor Bi L

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