NF-κB addiction and resistance to 5-fluorouracil in a multi-stage colon carcinoma model

Maria I. Körber, Simone Klingebrenner, Rupert Bartsch, Günther G. Steger and Robert M. Mader

Clinical Division of Oncology, Department of Medicine I, Comprehensive Cancer Center of the Medical University of Vienna, Vienna, Austria

Background

Colorectal cancer is one of the most frequently diagnosed malignancies in women and men. All systemic treatment options for colorectal cancer are based on 5-fluorouracil (5-FU), often administered in combination with oxaliplatin or irinotecan. Resistance to chemotherapy is a major obstacle to successful therapy.

Nuclear factor kappa light chain enhancer of activated B cells (NF-κB) is a collection of heterodimeric transcription factors. Activation of NF-κB enables cells to protect themselves from environmental stress and its misregulation is associated with chronic inflammation and cancer-related phenomena such as proliferation, cell survival and cell invasion [1]. In colorectal cancer, NF-κB inhibition may overcome resistance against chemotherapy as well as enhance the cytotoxicity of drugs [2, 3, 4].

Physiologically, NF-κB is kept inactive in the cytoplasm by a family of NF-κB-binding proteins called the inhibitors of NF-κB (IκB). Activation occurs via two different pathways: the non-canonical and the canonical pathway. Upon activation, NF-κB inhibitors are phosphorylated and subsequently ubiquitinated and degraded via the proteasome. Released NF-κB subunits translocate into the nucleus and control expression of hundreds of genes.

In addition to the canonical activation of NF-κB, Tpl2 (also known as Cot) activates NF-κB when overexpressed. Tpl2, a serine/threonine kinase, activates ERK-1/2 MAP kinases through a MEK1 pathway. Interestingly, all Tpl2 is stoichiometrically bound to NF-κB1/p105 in unstimulated cells and is thereby inactive [5]. It is now well known, that the activation of Tpl2, as in the case of other MAP3 kinases, is regulated by phosphorylation at Thr290 and S400.

Study aim

In this study, we investigated 1) the influence of NF-κB on resistance to 5-FU by inhibition of several steps in the NF-κB pathway using seven inhibitors and 2) we investigated the role of Tpl2 in a multi-stage colon cancer model in vitro.

Material and methods

To investigate the influence of NF-κB on chemoresistance we used a multi-stage colon cancer model consisting of four cell lines: the native line CCL227 (lymph node metastasis from a primary colon carcinoma) and the three resistant subclones (low, intermediate and high resistant phenotype). The subclones were produced by continuous exposure of tumor cells to increasing concentrations of 5-FU as described previously [6].

To establish dose-response relationships, cells were exposed to increasing concentrations of different inhibitors to determine the inhibitory concentration of 50% of the cells (IC50) using an MTT-Assay (CellTiter 96® Non-Radioactive Cell Proliferation Assay, Promega). Six different inhibitors of NF-κB, 3 of them mainly inhibiting phosphorylation (BAY 1170-82, violacein, lupeol), 2 blocking the proteasome (disulfiram, MG-132) and 1 specifically inhibiting the nuclear import (SN-50). Additionally, we also used an inhibitor of Tpl2/Cot. Data obtained from these experiments were fitted to a dose-response curve to
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define the corresponding IC$_{50}$ concentration (GraphPad Prism, version 4).

Results

Considering the IC$_{50}$ of the parent cell line CCL227, the most effective drugs were Tpl2-inhibitor (IC$_{50}$: 0.004 µM), MG-132 (IC$_{50}$: 0.2 µM), BAY 1170-82 (IC$_{50}$: 0.6 µM), and disulfiram (IC$_{50}$: 1.8 µM). Among these compounds, Tpl2-Inhibitor and BAY 1170-82 did completely overcome resistance, showing even lower IC$_{50}$ concentrations in the resistant phenotypes when compared with CCL227 (Figure 1). Being less effective in the native cell line, violacein (IC$_{50}$: 168 µM) and lupeol (IC$_{50}$: 58 µM) were nevertheless able to circumvent resistance at all resistance levels.

Remarkably, we measured the lowest IC$_{50}$ at 0.002 µM using Tpl2-Inhibitor. By blocking NF-κB via this non-canonical pathway we were able to overcome resistance totally at nanomolar levels of the compound being the high resistant cell line the most sensitive of all (0.004 µM for CCL227, 0.007 µM for +5 µM FU, 0.004 for +25 µM FU and 0.002 µM for +125 µM FU).

Blocking the proteasome via MG-132 and disulfiram led to similar results with both drugs able to overcome resistance partially (IC$_{50}$ concentrations in the nanomolar range). These compounds were at least ten times more effective than the inhibitors of phosphorylation, violacein and lupeol. Independent of the degree of resistance, cells were insensitive to SN-50, which blocks the intracellular transport of the p50 subunit of NF-κB (IC$_{50}$ > 100 µM).

Conclusions

Our results suggest that NF-κB has a pivotal impact on the viability of the native colon carcinoma cell line CCL227. All NF-κB inhibitors except SN-50 were able to inhibit growth and to cause cell death in vitro. Our data not only indicate that NF-κB has an effect on the viability of native cells, but is also relevant for resistance against 5-FU, as we were able to overcome resistance in the low, the intermediate and the high resistant phenotype. Some of the inhibitors (e.g., Tpl2 inhibitor and BAY 1170-82) showed even lower IC$_{50}$ concentrations in resistant phenotypes indicating an NF-κB addiction in these cell lines. The efficacy of Tpl2 inhibition in our model potentially uncovers a completely new mechanism to circumvent chemoresistance in colon cancer. With regard to cancer treatment, there is little data on the potency of Tpl2 inhibition so far. In summary, our data suggest that this targeted approach may be a strategy, which should be investigated in further studies.

References

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