



©2011 Dustri-Verlag Dr. K. Feistle
ISSN 0946-1965

DOI 10.5414/CP49069

Suicide activation in a 5-fluorouracil resistant colon cancer model in vitro*

R.M. Mader¹, M. Kalipciyan¹, P. Ohana², A. Hochberg² and G.G. Steger¹

¹Dept. of Medicine I, Medical University of Vienna, Vienna, Austria, and

²Dept. of Biological Chemistry, Hebrew University of Jerusalem, Jerusalem, Israel

Key words

tumor specific suicide system – atoxic compounds – cytotoxic agents – tumor selective gene therapy – chemoresistant colon cancer in vitro

*This extended abstract summarizes a poster presentation given by R.M. Mader during the 8th Annual Meeting 2010 of the Central European Society for Anticancer Drug Research (CESAR) held in St. Gallen, Switzerland, July 01 – 03, 2010.

Background

One of the limitations of all current therapies is a problem well-known to clinical oncologists: although an efficient agent for therapy is available, the specific distribution from the peripheral blood circulation to the malignant lesion is hardly selective. As a consequence, only a small percent of the administered dose distributes into the tumor, whereas the remaining drug causes unwanted side effects. One of the possible approaches to enhance the specificity of tumor therapy is gene therapy based on suicide genes exploiting tumor specific activation mechanisms [1]. Moreover, this approach may also be a valid alternative therapeutic strategy to overcome drug resistance by activation of atoxic compounds to cytotoxic agents.

Study aim

Aim of this project was the in vitro evaluation of tumor specific suicide systems exploiting tumor selective gene therapy techniques in a chemoresistant colon cancer model in vitro.

Methods

Adenoviral cosmids driving the suicide gene cytosine deaminase were generated to convert the prodrug 5-fluorocytosine (5-FC) to the toxic compound 5-fluorouracil (5-FU). To ensure specificity of drug activation, cytosine deaminase was selectively expressed and enhanced intracellularly under the control of the tumor associated promoters CEA and H19 [2, 3] after recombination via Cre/loxP-sequences in trans, thus preventing the unwanted expression of cytosine deaminase in normal cells. To achieve this, suicide plasmids were generated in *E. coli* using pIRESneo2 (Clontech) and subsequently transferred to the adenoviral cosmid pAxcw (RIKEN). After

packing with λ -bacteriophages (Promega), cosmid DNA was isolated and transfected with the COS-TPC method in the cell line 293 as feeder cell line to obtain high-titre adenoviral cosmids for experimental purposes. After multiplication, isolation and control of the cosmids, the colon cells CCL227 and the resistant subclones were transfected using different viral titers and incubation periods. The activation of the Cre/loxP-system was then achieved in trans by co-infection of Cre recombinase (under the control of the promoters of CEA or H19) concomitantly with the suicide gene cytosine deaminase under control of the very strong CAG promoter [4]. Control plasmids were investigated in parallel as positive and negative controls.

To establish dose-response curves, transfected cells were incubated with 5-FC (4 – 7 days), which was converted intracellularly to 5-FU by selective expression of the enzyme cytosine deaminase. In these experiments, HeLa cells (CCL-2) devoid of CEA expression were used as negative controls.

The cytotoxic effect of 5-FC was assessed in a primary adenocarcinoma of the colon, its lymph node metastasis, and different chemoresistant subclones in vitro, which has previously been described in detail [5, 6]

Results

A prodrug activation of near 100%, was observed in vitro with constitutive suicide gene expression compared with the parent drug 5-FU. After cell specific conversion of 5-FC to 5-FU by CEA or H19-driven suicide systems, these results were attenuated indicating a strong dependence on the promoter activity in our cellular system. Chemoresistance was partially circumvented, particularly in the low and intermediate resistance phenotypes. Although H19 was expressed to a lesser degree in some normal cells, the efficiency obtained by H19-driven suicide systems was half of that of the parent drug 5-FU, but was remarkably higher when compared

Correspondence to
Prof. Dr. R. Mader
Dept. of medicine 1,
Medical University of
Vienna, Department of
Clinical Oncology,
Währinger Gürtel 18 –
20, 1090 Vienna, Austria
robert.mader@
meduniwien.ac.at

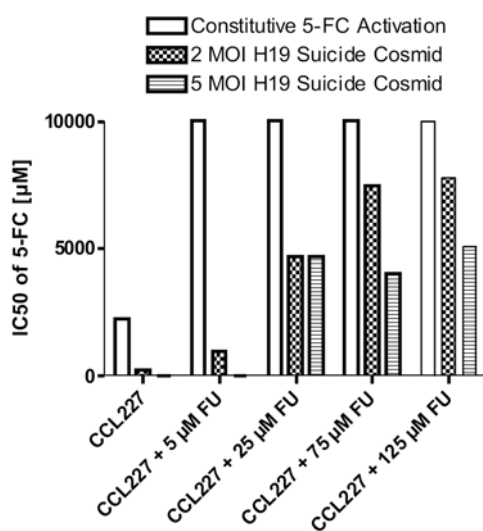


Figure 1. Amplification of cytotoxicity achieved by using a selective H19 promoter to convert FC to FU intracellularly. IC₅₀ < 10,000 µM was capped in the graph; MOI = Multiplicity of infection. Cell lines used were generated from naïve CCL227 by continuous exposure to FU at increasing concentrations (acquired drug resistance phenotype).

with the constitutive activation of 5-FC via a control cosmid, where IC₅₀ < 10,000 µM were often observed (Figure 1). These figures were further increased at higher viral titers (2 vs. 5 MOI) with the best results obtained in the low resistance phenotype (CCL227 continuously exposed to 5 µM FU). As all our resistant subclones were generated by continuous exposure to FU in order to mimic an acquired drug resistance phenotype, the results obtained reflect a difficult setting similar to clinical progression under therapy.

Conclusion

Besides the feasibility of the chosen approach, these data indicate the constraints of the infection system. Due to the non-specific toxicity of adenoviruses, optimal virus titers around 200 – 300 MOI can hardly be achieved with the actual adenoviral systems, as titers above 50 MOI showed overt toxicity in our model. When available, gene delivery systems with a 10-fold lower intrinsic toxicity would be an excellent opportunity for further translation of tumor specific suicide systems for clinical applications. The data obtained with the H19 promoter clearly indicate that intracellular amplification of the signals generated via H19-driven suicide cosmids are superior to constitutive and linear activation of 5-FC by a factor up to 10, particularly in the low resistance phenotype.

Acknowledgment

This work was sponsored by a grant of the Jubiläumsfonds der Österreichischen Nationalbank (project nr. 11960).

References

- [1] Ueda K, Iwahashi M, Nakamori M et al. Carcino-embryonic antigen-specific suicide gene therapy of cytosine deaminase/5-fluorocytosine enhanced by the Cre/loxP system in the orthotopic gastric carcinoma model. *Cancer Res.* 2001; *61*: 6158-6162.
- [2] Ariel I, deGroot N, Hochberg A. Imprinted H19 gene expression in embryogenesis and human cancer. *Am J Med Genetics.* 2000; *91*: 46-50.
- [3] Ohana P, Bibi O, Matouk I, et al. Use of H19 regulatory sequences for targeted gene therapy in cancer. *Int J Cancer.* 2002; *98*: 645-650.
- [4] Niwa H, Yamamura KI, Miyazaki JI. Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene.* 1991; *108*: 193-200.
- [5] Mader R, Sieder A, Braun J et al. Transcription and activity of 5-fluorouracil converting enzymes in fluoropyrimidine resistance in colon cancer in vitro. *Biochem Pharmacol.* 1997; *54*: 1233-1242.
- [6] Schmidt WM, Kalipciyan M, Dornstauder E et al. Dissecting progressive stages of 5-fluorouracil resistance in colon cancer using GeneChip RNA expression profiling. *Int J Cancer.* 2004; *112*: 200-212.