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Expression Pattern of Several Cytochrome P450 Iso-enzymes and Multi-drug Resistance Genes in Pancreatic Cancer Cell Lines in Response to the Chemotherapeutic Agents Gemcitabine and 5-Fluorouracil

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In the present study, the expression pattern of some of Cytochrome P450 isoforms **(CYP1A1, 1A2, 3A4, 2B6, 2C8-19, 2D6,** and **2E1)** was investigated in vitro, primary (HD259 and HD264) and commercial (BxPc3 and T3M4) pancreatic cancer cell lines, in relation to their exposure to the chemotherapeutic agents 5-FU and gemcitabine. In addition, this study assessed the expression of some Multi-drug resistance genes **(MDR1, MDR3, MRP1, MRP2, MRP3** and **MRP5)** in the above mentioned pancreatic cancer cell lines, again in response to their treatment with 5-FU and gemcitabine. It was our goal to identify the Cytochrome P450 isoenzymes and the Multi-Drug Resistance Genes which might play a role in the metabolism of the two above-mentioned anticancer drugs, and whether the pattern of expression of certain CYPs and MDR's in different pancreatic cancer cell lines contributes to the difference of response of those cell lines to 5-FU and gemcitabine. This knowledge could help to identify patients who may benefit from a specific anticancer drug and who may not.

The cell lines were treated with either 5-FU or gemcitabine for a defined period of time, followed by a recovery period without treatment. The treatment and recovery phases were repeated one more time. At the end of each period cells were collected and processed for RNA and protein extraction. Untreated controls were investigated at the same time points. The length of the treatment and recovery phases were adapted to the different doubling times of the commercial and primary cell lines. Therefore the study design varied for both primary and commercial cell lines in the duration of drug exposure and drug-free periods.

In order to exclude a possible early induction of the drug-metabolizing enzymes, two of our pancreatic cancer cell lines, the primary cell line HD264 and the commercial cell line T3M4, were investigated for their enzyme induction at the mRNA level during the first 24 hours after chemotherapy exposure. The chemotherapy concentration used in this experiment is the same as the one used for the long-term experiment.

There is a difference in the rate of growth between the primary and commercial cell lines. The progressive slowness in the rate of cell growth of the primary cell lines lead to their longer doubling

times. Cell counting, as a technique, seemed to be the more accurate method than MTT in assessing the doubling time.

In regard to the effect of the chemotherapeutic agents, 5-FU exhibited a stronger lethal effect on both primary and commercial cell lines. The impact of 5-FU was even more remarkable on the commercial cell lines.

Concerning the induction of different Cytochrome P450 isoenzymes, it was more frequent in the commercial cell lines than in the primary cell lines. The induction of **CYP1A1** in the 5-FU-treated BxPc3 cells was, by far, the most striking and strongest induction in the whole study. It remains a possibility that **CYP1A1** was behind the activation of 5-FU and consequently to its remarkable lethal effect on the cell lines BxPc3 and T3M4 cells. **CYP2E1** and **CYP2D6** were the most frequently induced isoenzymes in all cell lines we investigated. **CYP2E1** was the most frequently induced CYP in the primary cell lines and **CYP2D6** in the commercial cell lines. **CYP1A2** and **CYP2B6** were expressed in all four cell lines we studied but were not induced in any of them by chemotherapy. **CYP2C8-19**, however, was the only lacking enzyme, as it was not expressed in both primary pancreatic cell lines.

In reference to the Multi-Drug Resistance Genes, MRP3 was the only MRP member expressed in both primary and commercial cell lines. It was evident that 5-FU inhibited the expression of MRP3 and, at the same time, could generate its extensive lethal effect on those cells. In the commercial cell lines, MRP3 was over-expressed after gemcitabine and under-expressed after 5-FU treatment which could explain the remarkable resistance of those cell lines to gemcitabine and their immense sensitivity to 5-FU. In respect of MRP1 and MRP5, they were both expressed in the primary cell lines. In one primary cell line namely HD264, those two genes could be inhibited by 5-FU and activated by gemcitabine, which is correspondent to the higher lethal effect of 5-FU and the lower lethal effect of gemcitabine. On the other hand, MDR1, MDR3 and MRP2 were not significantly expressed in the pancreatic cancer cell lines we studied.