The Role of DNA Topoisomerase II In Drug Resistance and Sensitivity.

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SUMMARY AND CONCLUSIONS

Intrinsic or acquired resistance of human tumors to chemotherapy is often multifactorial. One of the factors involved in resistance is the nuclear enzyme DNA topoisomerase II (TopoII) which is known to be the target of a variety of cytotoxic drugs which are widely used in the clinic. A factor complicating TopoII research is the presence of two TopoII isoforms in human cells which are called TopoII*α* and ß. In this thesis the role of these isoforms in resistance in small cell lung carcinoma cell line models is examined and the regulation of TopoII expression in human ovarian tumors is investigated.

In **chapter 1** recent literature on human DNA topoisomerase II*α* and ß and their roles in chemotherapy resistance is extensively reviewed.

Chapter 2 describes the overexpression of the multidrug resistance associated protein (MRP), drug accumulation defects and downregulation of TopoII*α* and ß mRNA and protein levels with increasing adriamycin resistance development in GLC_4 sublines. All of these resistance mechanisms were already activated in the cell line with the lowest resistance factor (2-fold) which may be clinically relevant. In a partial revertant (stable 10-fold resistant) of the most resistant subline (which was 150-fold resistant), the expression of the described resistance mechanisms was intermediate. Interestingly, TopoII*α* and ß levels were decreased similarly in the different sublines despite the fact that these isozymes are regulated differently during the cell cycle. In this cell line panel the decreases in both isozymes apparently contribute to adriamycin resistance even at clinically relevant resistance levels.

The mechanism behind the downregulation of TopoII*α* mRNA levels in the cell line panel found in chapter 2 was analyzed in **chapter 3**. No changes in TopoII*α* mRNA half-life, nor genetic rearrangements in the TopoII*α* gene were found in the most resistant subline. Fluorescence in situ hybridization using a TopoII*α* gene specific probe showed that the parental cell line GLC_4 contained a large subpopulation of cells with three TopoII*α* gene copies and a minor subpopulation with only two TopoII*α* gene copies. During adriamycin resistance development the latter subpopulation was selected. It was shown that the gene copy frequency per cell was in agreement with TopoII*α* mRNA- and protein levels. Therefore, it was concluded that gene dosage effects caused by selection for a certain subpopulation,

contributed importantly to the downregulation of TopoIIα mRNA- and protein levels during adriamycin resistance development in this cell line panel.

In **chapter 4** the development and characterization of VM26, mAMSA and mitoxantrone resistant sublines of GLC_4 was described. These cell lines were developed in order to study resistance mechanisms to TopoII-drugs other than adriamycin. In none of these cell lines overexpression of P-glycoprotein (P-gp) or MRP was detected, although in the mitoxantrone resistant subline a small mitoxantrone accumulation defect was found. The cell lines were cross resistant to other TopoII-drugs indicating atypical multidrug resistance (see chapter 1), which implicates TopoIIα or ß-related alterations as contributors to resistance development. The VM26 resistant subline showed a major decrease in TopoIIα protein (54% of the GLC_4 value) and the mAMSA resistant subline a decrease in TopoIIß protein (to 28%). In the mitoxantrone resistant subline the TopoIIα and ß protein levels were markedly decreased (TopoIIα to 31%, TopoIIß protein was undetectable). The decrease in TopoIIα protein in the VM26 and the mitoxantrone resistant sublines was mediated by decreased TopoIIα mRNA levels. Selection for cells with decreased TopoIIα gene copies numbers (from 3 to 2) contributes to the TopoIIα mRNA decrease in the VM26- and the mitoxantrone resistant sublines, just as was shown for the adriamycin resistant sublines of GLC_4 (chapter 3). In contrast to the findings for the adriamycin resistant cell lines, TopoIIα and ß mRNA and protein levels were decreased differently. No correlations were found for TopoIIß mRNA levels and resistance to VM26, mAMSA, fostriecin and mitoxantrone. Negative correlations were found for TopoIIα mRNA levels and resistance to the cleavable complex inducers VM26, mAMSA and mitoxantrone indicating that lower TopoIIα levels may contribute to resistance in the GLC_4 sublines. The positive correlation found for TopoIIα mRNA level and fostriecin resistance suggests that cells with lower TopoIIα levels are more sensitive to this TopoII activity inhibitor.

A quantitative RT-PCR assay for the determination of TopoIIα mRNA levels was described in **chapter 5**. The assay allows quantitation of very low amounts of TopoIIα mRNA in total RNA. GLC_4 and two resistant sublines were used to validate the assay. The RT-PCR assay quantitates TopoIIα mRNA on picogram level starting with less than 1 μg total RNA. The results obtained by the RT-PCR assay were in agreement with results obtained by Northern blotting, Western blotting and the TopoII activity assay indicating that this assay is a useful technique in TopoII research

and TopoII-drug resistance studies.

Chapter 6 describes the characterization of ovarian tumor samples with regard to several TopoII-related parameters. A correlation was found for TopoII*α* mRNA levels and TopoII*α* protein levels, and there was almost a correlation observed for TopoIIß mRNA and protein levels. This suggests that in these tumors both assays may be used to quantify the level of each isozyme. Remarkably, TopoIIß mRNA levels correlated with overall TopoII activity, while TopoII*α* mRNA levels did not. This might implicate an important role for TopoIIß in ovarian tumors.

PERSPECTIVE

The cell line studies presented in this thesis show that already in cells with low resistance factors (which are probably clinically relevant) decreases in TopoII levels may be found. However, as resistance to TopoII drugs is multifactorial it is very hard to predict how a TopoII decrease contributes to resistance. An even more complicating finding was that the TopoII gene copy loss in the mitoxantrone resistant subline, which was in agreement with the degree of downregulation of TopoII protein, seemed to be caused by selection of cells displaying gross genetic rearrangements compared with the parental cell line. In these cells an entire chromosome 17q arm was deleted, which is known to carry several oncogenes and tumor suppressor genes, and changes in the level of these genes may also influence the resistance level of a cell. The exact contribution of a TopoII decrease to resistance may be determined in the future by downregulating TopoII levels using antisense or ribozyme techniques or by upregulating TopoII levels using gene transfection techniques.

A factor complicating TopoII research is the presence of two TopoII isozymes in human cells, each having different features. The expression of these isozymes is regulated differentially during the cell cycle and in resistant cells the expression of each isoform may be affected differently, depending on which drug is used. It is therefore necessary to gain more insight in how sensitive each isoform is for the TopoII inhibitors which are used in the clinic. This may be achieved by purification of each isoform and performing TopoII activity inhibition assays and band depletion assays with each isozyme. These techniques may shed more light on the importance of the TopoIIß isoform, on which only limited data are available. Additionally, it

cannot be ruled out that more TopoII isoforms will be found in the future.

The cell line models will give more insight in the role of TopoII changes in resistance development. However, whether the cell line results can be extrapolated directly to tumors is questionable. Therefore, human tumor material has to be screened and reliable assays have to be developed which are able to quantify TopoII levels even when only very little tumor material (e.g. fine needle biopsies) is available. When certain assays are not applicable to biopsy material, short term tumor cultures may have to be used. These assays will give more information on TopoII levels regarding tumor types, but also on TopoII status within each tumor type and within each tumor. Ultimately, it may become necessary to estimate the amount of each isozyme present within a tumor in order to predict which TopoII drugs may have to be used.

Also the genetic background of a tumor with respect to other genes may be important. When the tumor lacks the genetic material which is necessary for a cell to die from a certain drug according to a certain programmed cell death route (e.g. VP16 induces apoptosis), these drugs may be useless in this specific tumor.

Even more intriguing is the idea to change the tumor in such a way that it will die from TopoII drugs more efficiently. When in the future TopoII-specific transcription factors are found or genes whose products can modify TopoII activity, it may become possible to upregulate TopoII levels or activity in a tumor to make it more sensitive to TopoII cleavable complex inducers, or to downregulate TopoII levels to make it more sensitive to TopoII activity inhibitors. Of course, transfection with the TopoII genes themselves may already be sufficient for these purposes. Also the genetic background regarding cell death routes may be altered when tumor specific gene therapy becomes possible. An even more complicated strategy may involve stimulation of the processes which take place at DNA level (such as transcription and replication) which are probably causing DNA damage and cell death when TopoII molecules are fixed on the DNA after treatment with cleavable complex inducers.

Summarizing, the TopoII status of a tumor could be an important predictive factor for the sensitivity of a tumor for TopoII targeting drugs. However, the relative sensitivities of both TopoII isozymes are not known at present and have to be clarified. When TopoII-isozyme specific transcription factors are found, each isozyme may be specifically upregulated, thus enhancing the sensitivity of the tumor for specific TopoII drugs. Another

strategy involves the enhancement of TopoII activity. Finally, it may be attempted to stimulate the processes which are involved in cell death pathways, in order to enhance cell death rates even when TopoII levels are not affected.