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 TopoIIα and β. 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 IIα and β 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 TopoIIα and β mRNA and protein levels with increasing adriamycin resistance development in GLC4 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, TopoIIα and β 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 TopoIIα mRNA levels in the cell line panel found in chapter 2 was analyzed in chapter 3. No changes in TopoIIα mRNA half-life, nor genetic rearrangements in the TopoIIα gene were found in the most resistant subline. Fluorescence in situ hybridization using a TopoIIα gene specific probe showed that the parental cell line GLC4 contained a large subpopulation of cells with three TopoIIα gene copies and a minor subpopulation with only two TopoIIα 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 TopoIIα mRNA- and protein levels. Therefore, it was concluded that gene dosage effects caused by selection for a certain subpopulation, contributed importantly to the protein levels during adriamycin resistance.

In chapter 4 cell lines were developed which were resistant to mitoxantrone and mitoxantrone complex inducers VM26 and VM26B. Cell lines were developed which were resistant to mitoxantrone and one third of these resistant sublines were shown to be resistant to VM26, mitoxantrone and one third of these resistant sublines were shown to be resistant to VM26, mitoxantrone and one third of these resistant sublines were resistant to VM26B. The levels of TopoIIα decreased in these resistant sublines. The decrease in TopoIIα levels may contribute to the resistance to VM26, mitoxantrone and VM26B. In the mitoxantrone resistant subline, a correlation was found between the decrease in TopoIIα levels and the decrease in cell sensitivity to VM26B.

A quantitative PCR assay was used to determine low amounts of TopoIIα mRNA. In the mitoxantrone resistant subline, a correlation was found between the decrease in TopoIIα mRNA and the decrease in cell sensitivity to VM26B. The decrease in TopoIIα mRNA was not correlated with the decrease in cell sensitivity to VM26. The decrease in TopoIIα mRNA was not correlated with the decrease in cell sensitivity to VM26B. Therefore, it was concluded that cells with low TopoIIα mRNA levels were more sensitive to VM26B.

In chapter 4 cell lines were developed which were resistant to mitoxantrone and mitoxantrone complex inducers VM26 and VM26B. Cell lines were developed which were resistant to mitoxantrone and one third of these resistant sublines were shown to be resistant to VM26, mitoxantrone and one third of these resistant sublines were shown to be resistant to VM26B. The levels of TopoIIα decreased in these resistant sublines. The decrease in TopoIIα levels may contribute to the resistance to VM26, mitoxantrone and VM26B. In the mitoxantrone resistant subline, a correlation was found between the decrease in TopoIIα levels and the decrease in cell sensitivity to VM26B. The decrease in TopoIIα levels was not correlated with the decrease in cell sensitivity to VM26. The decrease in TopoIIα levels was not correlated with the decrease in cell sensitivity to VM26B. Therefore, it was concluded that cells with low TopoIIα mRNA levels were more sensitive to VM26B.
Summary, conclusions, and perspective

Topoisomerase IIα and IIβ were reviewed. The role of these isoforms in human ovarian tumors is not clear.

Multidrug resistance (MDR) and downregulation of adriamycin resistance mechanisms were cross-resistant to other TopoI-drugs other than adriamycin. No 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 TopoI-drugs indicating atypical multidrug resistance (see chapter 1), which implicates TopoIα or β-related alterations as contributors to resistance development. The VM26 resistant subline showed a major decrease in TopoIα protein (54% of the GLC4 value) and the mAMSA resistant subline a decrease in TopoIβ protein (to 28%). In the mitoxantrone resistant subline TopoIα and β protein levels were markedly decreased (TopoIα to 31%, TopoIβ protein was undetectable). The decrease in TopoIα protein in the VM26 and the mitoxantrone resistant sublines was mediated by decreased TopoIα mRNA levels. Selection for cells with decreased TopoIα gene copies numbers (from 3 to 2) contributes to the TopoIα mRNA decrease in the VM26 and the mitoxantrone resistant sublines, just as was shown for the adriamycin resistant sublines of GLC4 (chapter 3). In contrast to the findings for the adriamycin resistant cell lines, TopoIα and β mRNA and protein levels were decreased differently. No correlations were found for TopoIβ mRNA levels and resistance to VM26, mAMSA, fostriecin and mitoxantrone. Negative correlations were found for TopoIα mRNA levels and resistance to the cleavable complex inducers VM26, mAMSA and mitoxantrone indicating that lower TopoIα levels may contribute to resistance in the GLC4 sublines. The positive correlation found for TopoIα mRNA level and fostriecin resistance suggests that cells with lower TopoIα levels are more sensitive to this TopoI activity inhibitor.

A quantitative RT-PCR assay for the determination of TopoIα mRNA levels was described in chapter 5. The assay allows quantitation of very low amounts of TopoIα mRNA in total RNA. GLC4 and two resistant sublines were used to validate the assay. The RT-PCR assay quantitates TopoIα 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 TopoI activity assay indicating that this assay is a useful technique in TopoI research.
and Topoll-drug resistance studies.

Chapter 6 describes the characterization of ovarian tumor samples with regard to several Topoll-related parameters. A correlation was found for Topollα mRNA levels and Topollα protein levels, and there was almost a correlation observed for Topollβ mRNA and protein levels. This suggests that in these tumors both assays may be used to quantify the level of each isozyme. Remarkably, Topollβ mRNA levels correlated with overall Topoll activity, while Topollα mRNA levels did not. This might implicate an important role for Topollβ 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 Topoll levels may be found. However, as resistance to Topoll drugs is multifactorial it is very hard to predict how a Topoll decrease contributes to resistance. An even more complicating finding was that the Topoll gene copy loss in the mitoxantrone resistant subline, which was in agreement with the degree of downregulation of Topoll 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 Topoll decrease to resistance may be determined in the future by downregulating Topoll levels using antisense or ribozyme techniques or by upregulating Topoll levels using gene transfection techniques.

A factor complicating Topoll research is the presence of two Topoll 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 Topoll inhibitors which are used in the clinic. This may be achieved by purification of each isoform and performing Topoll activity inhibition assays and band depletion assays with each isozyme. These techniques may shed more light on the importance of the Topollβ isoform, on which only limited data are available. Additionally, it cannot be ruled out that the cell line studies of changes in resistance factors may not be extrapolated to the clinical setting, which may have implications for the material (e.g. which is not necessarily applicable).

SUMMARY

Also the Topoll gene copy number may be important in determining resistance to Topoll drugs. It is necessary for the research to be extrapolated to the clinical setting, which may be more complicated.

Even more complicating is that it will die within a specific time frame, Topoll activity inhibition may be used to determine resistance to Topoll therapy in a tumor type, or to determine the activity of a drug. The activity of a drug may already be determined before a specific transfection becomes useless in the clinic. In some cases, death where a transfection is not applicable.

Summarized, the Topoll β isoform may already be used to determine resistance to Topoll therapy.
Summary, conclusions, and perspective

cannot be ruled out that more Topoll isoforms will be found in the future. The cell line models will give more insight in the role of Topoll 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 Topoll 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 Topoll levels regarding tumor types, but also on Topoll 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 Topoll 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 Topoll drugs more efficiently. When in the future Topoll-specific transcription factors are found or genes whose products can modify Topoll activity, it may become possible to upregulate Topoll levels or activity in a tumor to make it more sensitive to Topoll cleavable complex inducers, or to downregulate Topoll levels to make it more sensitive to Topoll activity inhibitors. Of course, transfection with the Topoll 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 Topoll molecules are fixed on the DNA after treatment with cleavable complex inducers.

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