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

Withoff, Sebo

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Document Version Publisher's PDF, also known as Version of record

Publication date: 1996

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA): Withoff, S. (1996). The Role of DNA Topoisomerase II In Drug Resistance and Sensitivity. s.n.

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Chapter 7

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 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 $II\alpha$ 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 Topoll α 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, Topoll α 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 Topoll*a* mRNA levels in the cell line panel found in chapter 2 was analyzed in **chapter 3**. No changes in Topoll*a* mRNA half-life, nor genetic rearrangements in the Topoll*a* gene were found in the most resistant subline. Fluorescence in situ hybridization using a Topoll*a* gene specific probe showed that the parental cell line GLC₄ contained a large subpopulation of cells with three Topoll*a* gene copies and a minor subpopulation with only two Topoll*a* 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 Topoll*a* mRNA- and protein levels. Therefore, it was concluded that gene dosage effects caused by selection for a certain subpopulation, contributed importatein levels during a

In chapter 4 and mitoxantrone lines were develop drugs other than a P-glycoprotein (P-g resistant subline a cell lines were cr multidrug resistanc alterations as cont subline showed a value) and the mA 28%). In the mitoxa were markedly dec ble). The decrease resistant sublines lection for cells wit contributes to the trone resistant su sublines of GLC, resistant cell lines, differently. No cor tance to VM26, m were found for Tc plex inducers VM2 la levels may con correlation found that cells with low inhibitor.

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contributed importantly to the downregulation of Topolla 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 GLC₄ was described. These cell lines were developed in order to study resistance mechanisms to Topolldrugs 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 Topoll-drugs indicating atypical multidrug resistance (see chapter 1), which implicates Topolla or β -related alterations as contributors to resistance development. The VM26 resistant subline showed a major decrease in Topolla protein (54% of the GLC_4 value) and the mAMSA resistant subline a decrease in Topollß protein (to 28%). In the mitoxantrone resistant subline the Topolla and B protein levels were markedly decreased (Topolla to 31%, Topollß protein was undetectable). The decrease in Topolla protein in the VM26 and the mitoxantrone resistant sublines was mediated by decreased Topolla mRNA levels. Selection for cells with decreased Topolla gene copies numbers (from 3 to 2) contributes to the Topolla 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, Topolla and ß mRNA and protein levels were decreased differently. No correlations were found for Topollß mRNA levels and resistance to VM26, mAMSA, fostriecin and mitoxantrone. Negative correlations were found for Topolla mRNA levels and resistance to the cleavable complex inducers VM26, mAMSA and mitoxantrone indicating that lower Topolla levels may contribute to resistance in the GLC₄ sublines. The positive correlation found for Topolla mRNA level and fostriecin resistance suggests that cells with lower Topolla levels are more sensitive to this Topoll activity inhibitor.

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

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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 Topoll α mRNA levels and Topoll α protein levels, and there was almost a correlation observed for Topoll β mRNA and protein levels. This suggests that in these tumors both assays may be used to quantify the level of each isozyme. Remarkably, Topoll β mRNA levels correlated with overall Topoll activity, while Topoll α mRNA levels did not. This might implicate an important role for Topoll β 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 Topoll is isoform, on which only limited data are available. Additionally, it

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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 Topollspecific 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

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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.

SAMEN

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