Abstract

DNA topoisomerase II is required in a number of independent roles in the DNA metabolism particularly, in mitosis. The enzyme is able to alter the topology of DNA by introducing transient doublestrand breaks into DNA and passing an intact DNA helix through the opening before resealing the break. In mammalian cells two closely related but genetically distinct isoforms of topoisomerase II (α and β) are expressed. Unique functions for each of the two isoforms have been suggested by their differences in cell cycle expression profile, in subcellular localizations and in chromatin binding at mitosis. However, at mitosis the exact functions of DNA topoisomerase IIα and β are at the moment not fully understood. In this thesis I had the opportunity to work with a human cell line H69-VP which carries a homozygeous mutation of the nuclear localization sequence (NLS) of DNA topoisomerase IIα. This H69-VP cell line expresses the topoisomerase IIα outside the nucleus. In these cells topoisomerase IIβ showed a normal nuclear localization whereas at mitosis it diffused away from the chromatin despite the nuclear lack of the IIα- form. In 80% of the cells chromosome condensation and disjunction was performed with the aid of cytosolic topoisomerase IIα which bound to the mitotic chromatin with low affinity. Consequently an increased rate of nondisjunction is observed in these cells. In 20% of the mutant cells neither of the isoforms were bound to the mitotic chromatin which appeared as an unstructured DNA spheroid unable to undergo disjunction and cytokinesis.

In the parental cell H69-WT both isoforms are expressed inside the nucleus. At mitosis in these cells the topoisomerase IIβ diffused away from the chromatin and the topoisomerase IIα bound the mitotic chromatin with high affinity. The genotype was mostly diploid and

stable. It is concluded that high affinity chromatin binding of topoisomerase IIα is essential for chromosome condensation/disjunction and that topoisomerase IIβ does not adopt these functions.

In tumour cells a centrosomal protein was recognized by three distinct epitopes of human topoisomerase IIα. The protein associated at the centrosomes differs in many aspects compared to the normal nuclear topoisomerase IIα. The topoisomerase IIα-like protein resembles a modified form of topoisomerase IIα with an apparent size of 205 kDa compared to 170 kDa. It is unclear which additional mechanisms give rise to a changed electrophoretic mobility but the centrosomal protein is neither a splice variation nor hyperphosphorylated form of topoisomerase IIα. The expression of the protein is constant in all stages of the cell cycle and it appears in proliferating as well as in resting cells. It may or may not exhibits topoisomerase II activity. I can therefore only speculate what kind of function the protein may have. In this form is it unlikely that the centrosomal protein is involved in catalytical turnover. However, it is definitely stored at the centrosome. If there is not sufficient topoisomerase IIα present at mitosis the centrosomal proteins might adopt the function and a mitotic catastrophe in the cells could therefore be prevented.