

Contents

1	Introduction	3
1.1	DNA-topology.....	3
1.2	DNA topoisomerases	5
1.3	Type II.....	6
1.4	Human topoisomerase II	8
1.5	Structural and functional domains of human topoisomerase II.....	10
1.6	Human topoisomerase II in various stages in cell cycle.....	11
1.7	Regulation and control of human topoisomerase II.....	12
1.8	Enzymatic mechanism for human topoisomerase II.....	14
1.9	Subcellular localization of human topoisomerase II.....	17
1.10	Inhibitors of the topoisomerase catalytical cycle.....	19
2	Formulation of questions.....	20
3	Methods and materials	21
3.1	Cell lines.....	21
3.2	Lymphocytes isolation	22
3.3	Cell counting	23
3.4	Cell cycle analysis.....	24
3.5	Drugs	24
3.6	Antibodies	24
3.7	Topoisomerase II α standard.....	25
3.8	Indirect immunofluorescence microscopy	25
3.9	Pre-absorption of antibodies in immunofluoresence	28
3.10	Decatenation.....	28
3.11	Agarose gelelectrophoresis	28
3.12	Extract for decatenations assay	29
3.13	Immuno-band depletion assay	29

3.14	Biochemical methods for revealing subcellular organization of topoisomerase II.....	30
3.15	Salt-extraction of chromatin bound topoisomerase II.....	31
3.16	Centrosome isolation.....	32
3.17	Phosphatase treatment.....	32
3.18	TCA- precipitation	33
3.19	Samples for SDS-PAGE gel	33
3.20	SDS-Polyacrylamid gel electrophoresis	33
3.21	Western blot	34
3.22	Immunodetection for Western blot	35
3.23	RNA isolation and reverse transcription.....	35
3.24	Statistics	38
4	Results	38
4.1	Essential Mitotic functions of DNA topoisomerase II α are not adopted by topoisomerase II β in human H69 cells.....	38
4.2	Extracellular expression of active topoisomerase II α	39
4.2.1	The subclone H69/VP with a deletion in the NLS sequence.....	39
4.2.2	Enzyme activity in whole cell lysate.....	40
4.2.3	Enzyme levels in whole cell lysate	40
4.2.4	Enzyme localization in cytosolic and nuclear fraction	41
4.2.5	Immunoband depletion.....	41
4.3	Chromosome condensation and disjunction	43
4.3.1	Cellular localization of topoisomerases in H69-WT	44
4.3.2	Cellular localization of topoisomerases in H69-VP	45
4.3.3	Binding affinity of topoisomerases to chromatin.....	47
4.3.4	DNA mass distribution.....	49
4.4	DNA-topoisomerase II α like protein is associated with centrosomes.....	50

4.4.1	Several domains of topoisomerase II α co-localize with γ -tubulin.....	50
4.4.2	Topoisomerase II α co-localize with γ -tubulin in A431	50
4.4.3	Topoisomerase II α co-localize with γ -tubulin in lymphocytes	51
4.4.4	Topoisomerase II α - like protein of 205 kDa in the cytosolic fraction.....	52
4.4.5	The topoisomerase II α -like protein is present in isolated centrosomes.....	53
4.4.6	The centrosomal protein is not a splice variant of normal topoisomerase II α	54
4.4.7	The centrosomal protein is not a hyperphosphorlated form of normal topoisomerase II α	55
4.5	The centrosomal protein may not exhibit a type II activity.....	56
5	Discussion	56
5.1	Essential mitotic functions of topoisomerase II α are not adopted by the β -form.....	56
5.2	Role of topoisomerase II α in chromosome condensation and disjunction	60
5.3	DNA-topoisomerase II α -like protein is associated with centrosomes.....	63
6	Abbreviations	68
7	Reference.....	69
8	Figures.....	80