DNA TOPOISOMERASES: A REVIEW ON THEIR FUNCTIONAL ASPECTS IN CELL DIVISION

Aditi Srivastava, Rumana Ahmad, A.N Srivastava*

*Department of Biochemistry, Department of Pathology**

Era's Lucknow Medical College & Hospital, Sarfarazganj Lucknow, U.P., India-226003

Received on : 27-03-2018
Accpected on : 21-05-2018

Address for correspondence
Dr. A.N. Srivastava
Department of Pathology
Era's Lucknow Medical College & Hospital, Lucknow-226003
Email: ans4csmmu@gmail.com
Contact No: +91-5223226777

ABSTRACT

DNA is double helical macromolecule which carries all the genetic information and it is usually found enveloped inside a nucleus. The DNA helix relaxes and supercoils itself frequently in order to derive information from the genes during processes like transcription, condensation, replication and recombination, which require mutable or immutable alterations to cause the separation of the two DNA strands. Due to problems caused by the helical structure of DNA, these topoisomerase enzymes perform the required DNA uncoiling. Their role in cell cycle is also significant as their mutation leads to failure of anaphase separation (1, 2). In the present review, the important roles of DNA topoisomerases and their inevitable role in cell growth and cell cycle are discussed viz. how they function in cell proliferation and what are the results when different inhibitors are added to the cells, affecting cell cycle at various checkpoints .

Keywords: Topoisomerases, DNA supercoiling, Cell division, Drug targets, Fluoroquinolones

INTRODUCTION

DNA is the most important macromolecule in cell biology. As the name suggests, it is the largest molecule in a cell containing almost thousands of genes. The size of the linear DNA is longer than the cell in which it is contained. Therefore, it is clear that it requires an elaborate and complex level of organization and compaction. The DNA must be tightly packed inside the nucleus and the packing must allow access to all the relevant information present on the DNA molecules in order to carry on all life processes. These life processes require momentary separation of the two strands of DNA, thus requiring something to relieve the helical stress of DNA (1). This property of the enzyme topoisomerase has been exploited for clinical benefits by different exogenous agents to interfere with cell proliferation (6).

DNA topoisomerases portray key roles in DNA replication, transcription, chromosome segregation, and recombination. Double stranded DNA can be loosely wound (negatively supercoiled) or tightly wound (positively supercoiled), depending on its lowest energy state, therefore causing DNA to bend and coil in space. These interconversions in the topology of DNA such as the knotting and unknotting of DNA, catenation and decatenation of DNA rings, including the formation of positive and negative supercoils are carried out by DNA topoisomerases. They calibrate the steady-state level of DNA by enabling protein interactions with DNA and restraining immense supercoiling which could be detrimental to life.

HISTORICAL PERSPECTIVE

James Wang in 1971 discovered the first DNA topoisomerase and named it ω-protein on its ability to relax negatively supercoiled DNA of bacteriophage λ. It is encoded by *topA* gene in *E. coli.* Later, Wang and Liu in 1979 renamed it as DNA topoisomerase I. A similar Type I topoisomerase activity has been isolated from *Salmonella typhimurium*, *Micrococcus luteus, Haemophilus gallinarum* (3), *S. typhimurium* (4), *Agrobacterium tumefaciens*, *Bacillus megaterium* (5), and *Bacillus stearothermophilus*. DNA topoisomerase I (Topo I) is known as type IA in prokaryotes and type IB in eukaryotes. A few years later, Martin Gellert and co-workers, unearthed an enzyme called DNA gyrase, in the course of searching for host co-factors that aided site-specific recombination by bacteriophage λ which were competent of introducing supercoils. Later it was named as DNA topoisomerase II (Topo II).

MECHANISM OF ACTION

A reaction catalyzed by DNA topoisomerases leads to changes in the Linking number (L). Linking number is the number of times one strand of DNA passes over the other strand. In B form of DNA, the linking number is 10.5. When ΔL increases, the helical pitch becomes tighter, resulting in positive supercoiling. A decrease in ΔL results in negative supercoiling. DNA topology can only be changed by incision and rejoining of DNA. The enzyme action is usually oriented towards ΔL=0, which is the most stable form of DNA (Fig.1.)

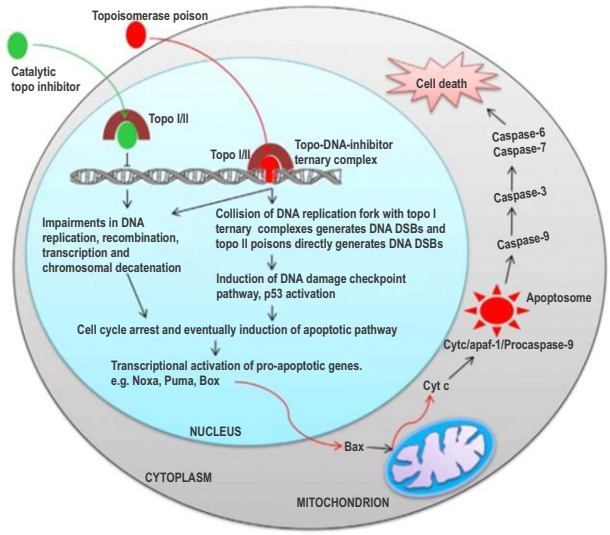

Fig.1. Mechanism of Action of Topoisomerases. Image courtesy: Jain et.al, 2017 (16)

They cleave single or double strands of DNA and finally reseal them, which involves the formation of a phosphodiester bond between one end of broken strand and a tyrosine residue present in the active site of DNA topoisomerase. Some of them require divalent metal ion co-factors.

CLASSIFICATION

There are two types of topoisomerases: type I (E.C. 5.99.1.2) which make single stranded cuts in the dsDNA and type II (E.C .5.99.1.3) which cut and pass both the strands of dsDNA. Major types of DNA topoisomerases have been listed in Table 1.

ENZYME	SOURCE	SIZE (kDa) AND SUBUNIT	FUNCTION
Top I(ω-protein)	Bacteria (*E.coli*)	97 Monomer	Relaxes supercoils
Eukaryotic Top I	Human	91 Monomer	Relaxes both positive and negative supercoils
Vaccinia virus Top I	Vaccinia virus	37 Monomer	ATP stimulated activity
Eukaryotic Top II (IIα & IIβ-isoforms)	Human	174 and 180 Homodimer (Heart like shape)	Relaxes, unknots, and decatenates closed circular DNA (ATP –dependent)
Prokaryotic Top IV	Bacteria(*E.coli*)	84 and 70(C_2E_2) Heterotypic tetramer *parC* and *parE* genes	Relaxes, but not supercoils, DNA, potent decatenation (ATP-dependent)
Topoisomerase VI	Archaea (*Sulfolobus Shibatae)*	45 and 6O A_2B_2	Relaxes, but not supercoils (ATP-dependent)
Prokaryotic DNA gyrase	Bacteria (*E.coli*)	90 and 97(A_2B_2) Heterotypic tetramer *gyrA* and *gyrB* genes	Introducesnegative supercoils (ATP-dependent)
T4 Topoisomerase	Bacteriophage T4	58,51 and 18 2 copies of each subunit	Relaxes, but not supercoils (ATP-dependent)
Reverse gyrase	Thermophilic Archaea (e.g.*Sulfolobus acidocaldarius*)	120 Monomer	Introducespositive supercoils and relaxes negative supercoils (ATP-dependent)

Table 1: Classification of Topoisomerases

FUNCTIONALASPECTS

DNA Topoisomerases are required during DNA replication, transcription and homologous recombination. They play an important role in cell cycle, thus leading to cell growth. During the initiation of replication in prokaryotes, DNA gyrase helps in negative supercoiling of DNA to initiate replication. As the elongation step continues, the DNA is continuously relaxed. DNA gyrase and Topo I regulate the superhelicity of DNA correspondingly.

DNA topoisomerase inhibitors are majorly of two types:

i. Topoisomerase poisons which inhibit the relegation step thus, stabilizing covalent enzyme-DNA complex.

ii. Catalytic topoisomerase inhibitors which prevent the binding of topoisomerases to DNA or its cleavage.

These enzymes are molecular targets for various naturally derived drugs having anticancer and antibacterial properties. In abnormal cells or cancer cells, rapid cell division occurs requiring higher topoisomerase activity. Camptothecin, a natural alkaloid isolated from the bark of a Chinese tree (*Camptotheca acuminata*) acts as a DNA topoisomerase I poison and stabilizes TOP1-DNA complex by inhibiting relegation step (6, 7, 8). This stabilization produces DNA lesions leading to apoptosis. Similarly, Etoposide inhibits DNA topoisomerase II (9).

Topo II is required for chromosome condensation and separation of intertwined DNA molecules; in addition it also relieves DNA supercoiling. It helps in maintaining the chromatin structure of interphase cells. In *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, Topo I null mutants are viable due to substitution of its cellular function by Topo II. Double mutation of phenotype Topo I and Topo II leads to immediate arrest of cell growth leading to mortality. Topo I play a key role in the developmental process of fruit flies and mice, therefore if their genes are knocked out, it results in death at an early stage of emnryogenesis, because of rapid cell turnover number at this stage. Therefore, *topo* I compromised individual can no longer catch up with the superhelical stress generated by the DNA replication. This would ultimately result in death at an early stage of embryogenesis. They are potential drug targets like camptothecins which inhibit DNA and RNA synthesis; arrest cell cycle in the G2 phase, elevate p53 level leading to destruction of Topo I enzyme and activate signal transduction to stop the cell cycle.

Austrobailignan-1, is a natural lignan isolated from *Koelreuteria henryi* Dummer plant and has anti-cancer property. From the experiments enlisted in

different reviews, it has been found that effect of this compound on cancer cell lines arrested cell cycle at G2/M phase by increasing the expression level of p21 and p27 cell cycle inhibitors and decreasing the expression of enzyme M-phase inducer phosphatase 3 (Cdc25C). This compound has been found to inhibit DNA relaxation activity of Topo I.

Topo II enzymes are important for cell proliferation, thus in rapidly dividing mammalian cells there is a dramatic increase in the level of topo II α at the G2/M phase. Therefore, they are good targets for cancer chemotherapy in which drugs like doxorubicin and etoposide cause the enzyme to be covalently bound on the DNA and form cleavable complex thus losing its activity (10). The expression level of this enzyme can decrease due to down regulation of its mRNA, instability of mRNA and destruction of Topo II protein. Topo III has role in suppression of mitotic recombination between repetitive sequences (11). Mutations in Topo III lead to shortening of telomeric repeats thus, slowing down cell proliferation (12).

Fluoroquinolones are synthetic antibiotics that interact with topo II-DNA complexes and hinder helix relegation resulting in the formation of double stranded breaks (13). In 40-50% breast cancer cases, it has been evaluated that DNA topoisomerase II α is usually co-amplified with human epidermal growth factor receptor 2 (HER2) oncogene, indicating that patients with HER2 positive breast cancer showed a marked clinical response to topo II-inhibitors (14). These inhibitors affect the activity of topoisomerases by hindering mitochondrial DNA synthesis, which induces mitochondrial injury, disorders in respiratory chain and reduction in the intracellular store of ATP ultimately reducing cell activity. This encourages the cell to undergo apoptosis due to cell cycle arrest in the S- and/or G2-M phases (15). Topo IV and DNA gyrase are molecular drug targets for commonly used quinolone antibiotics.

CONCLUSION

DNA topoisomerases are an essential class of enzymes required for interconversions between different topological isoforms of DNA to carry out critical life processes. They have indispensible roles in DNA metabolism in bacteria and other organisms. Besides studying their role in different cellular processes, their role as anticancer and antibacterial drug targets `is noteworthy. The synthetic topoisomerase inhibitors cause toxicity and side effects on normal cells. Therefore, more research is needed on the functional aspects of DNA topoisomerases and the need to discover natural products having anti-topoisomerase activity.

REFERENCES

1. Kato S, Kikuchi A. DNA Topoisomerase: The key enzyme that regulates DNA super structure. Nagoya J Med Sci. 1998; 61: 11-26.
2. Watson JD, Crick FH. Genetical implications of the structure of deoxyribonucleic acid. Nature. 1953; 171: 964-967.
3. Shishido K, Ando T. Purification and characterization of DNA-relaxing enzyme from Haemophilus gallinarium. Biochim Biophys Acta. 1979; 563:261-5.
4. Leroy Liu F, Chung-Cheng L, Bruce AM. Type II DNA Topoisomerases: Enzymes that can unknot a topologically knotted DNA molecule via a reversible double-strand break. Cell. 1980; 19: 697-707.
5. Tabary X, Moreau N, Dureuil C, Goffic LF. Effect of DNA gyrase inhibitors pefloxacin, five other quinolones, novobiocin, and clorobiocin on Escherichia coli topoisomerases I. Antimicrob Agents Chemother. 1987; 31:1925-1928.
6. Pommier Y. Drugging topoisomerases: lessons and challenges. ACS Chem Biol. 2013; 8:82-95.
7. Pommier, Y. DNA topoisomerase I inhibitors: chemistry, biology, and interfacial inhibition. Chem Rev. 2009; 109: 2894-2902.
8. Hsiang YH, Hertzberg R, Hecht S, Liu LF. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerases I. J Biol Chem. 1985; 260: 14873-14878.
9. Bailly C. Contemporary challenges in the design of topoisomerases II inhibitors for cancer chemotherapy. Chem Rev. 2012; 112:3611-3640.
10. Chen AY, Liu LF. DNA topoisomerases - essential enzymes and lethal targets. Annu Rev Pharmacol Toxicol. 1994; 34: 191-218.
11. Hanai R, Caron PR, Wang JC. Human TOP3: A single-copy gene encoding DNA topoisomerase III. Proc Natl Acad Sci. 1996; 93:3653-3657.
12. Aldred KJ, Kerns R.J, Osheroff N. Mechanism of quinolone action and resistance. Biochem. 2014; 53: 1565-1574.
13. Kim RA, Caron PR, Wang JC. Effects of yeast DNA topoisomerase III on telomere structure. Proc Natl Acad Sci. 1995; 92:2667-2671.
14. Arriola E, Marchio C, Tan DS, Drury SC, Lambros MB, Natrajan R, Rodriguez-Pinilla SM, Mackay A, Tamber N, Fenwick K. et al. Genomic analysis of the HER2/TOP2A amplicon in breast cancer and breast cancer cell

lines. Lab Invest. 2008; 88: 491-503.

15. Elmore S. Apoptosis: A review of programmed cell death. Toxicol Pathol. 2007;35: 495-516.

16. Jain CK, Majumder HK, Roychoudhury S. Natural Compounds as anticancer agents targeting DNA topoisomerases. Curr Genomics. 2017; 18:75-92.

How to cite this article : Srivastava A, Ahmad R, Srivastava A.N ., Dna Topoisomerases: A Review On Their Functional Aspects In Cell Division. EJMR2018;5(2):1-4