4 INDIRECT DNA DAMAGE BY DNA TOPOISOMERASE INHIBITORS

The discovery of DNA topoisomerases in the 1970s solved the topological problem posed by DNA replication. These enzymes are major elements in cellular life and the target of a plethora of antibiotics and antitumor compounds. Their inhibitors are among the most efficient inducers of apoptosis[51] and include some of the most widely used anticancer drugs.[52]

Topoisomerases regulate the three-dimensional geometry (topology) of DNA, leading to the interconversion of its topological isomers and to its relaxation. Identical loops of DNA having different numbers of twists are topoisomers—that is, molecules with the same formula but different topologies—and their interconversion requires the breaking of DNA strands. Regulation of DNA supercoiling is essential to DNA transcription and replication, when the DNA helix must unwind to permit the proper function of the enzymatic machinery involved in these processes.

Among the various topoisomerases,[53] we briefly discuss the roles of Top1 and Top2. In eukaryotic cells, Top1 breaks a single DNA strand, whereas Top2 breaks both strands and requires ATP hydrolysis and Mg^{2+} for full activity. Both proteins introduce transient single-strand breaks in the DNA molecule and store the energy gained during the cleavage reaction in a transient covalent linkage between DNA and a tyrosine of the protein in order to use it later for their ligase activity. The catalytic mechanism in both cases consists of two transesterification steps beginning with a nucleophilic attack of a DNA phosphodiester bond by a tyrosyl residue from the topoisomerase active site. The resulting covalent attachment of the tyrosine to the DNA phosphate is either at the 3′ end of the broken DNA, in the case of nuclear and mitochondrial enzymes Top1 and Top1mt, or at the 5′ end of the broken DNA for the other topoisomerases. These are known as the "cleavable complex" because they are transient and have easily reversible linkages, which formation permits the DNA relaxation. In the religation step, a hydroxyl group from deoxyribose attacks the previously formed tyrosine phosphate, and the end result is a DNA molecule that is chemically unchanged but closed in a different topology.

Topoisomerases are crucial for the several DNA functions that require the DNA to be unraveled, a process that generates tension and entanglement in DNA. Removing positive supercoils is required for replication and transcription progression; otherwise, their accumulation hinders the melting of the DNA duplex by helicases and consequently polymerase translocation along the DNA template.

On the other hand, topoisomerase poisons may induce genetic instability.[54] In this connection, some alarming studies have been published suggesting that maternal exposure to low doses of dietary Top2 poisons, including bioflavonoids such as genistein or quercetin, may contribute to the development of infant leukemia.[55]

4.1 TOPOISOMERASE I MECHANISM

Topoisomerase I is located in areas of active RNA transcription in order to release superhelical stress generated during mRNA synthesis. As previously mentioned, in the case of eukariotic Top1, a single strand is attacked and a 3′-phosphotyrosyl linkage is formed. Religation takes place through attack of the 5′ hydroxyl to the previously formed phosphate group (Figure 7.9).

After making a transient break ("nick") of a single strand of DNA, the DNA relaxation mechanism is originated by "controlled rotation" rather than by "strand passage." In other words, Top1 enzymes relax DNA by letting the 5′-hydroxyl end rotate around the intact strand (Figure 7.10).