Cancer, DNA Repair Mechanisms, and Resistance to Chemotherapy

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Soon after the introduction of daunomycin and cytosine arabinoside (i.e., cytarabine) in the early 1960's, several reports appeared examining the effects of these agents on bone marrow and peripheral blood cells (1,2). A surprisingly high percentage of normal cells, as many as 92% in some patients treated with daunomycin, had gross chromosomal damage. As we look back on these early studies, it is clear that a cell's capacity to repair DNA damage was overwhelmed by the therapeutic doses of chemotherapy used. Knowing how such damage can lead to DNA rearrangements and, in turn, to secondary leukemias, it is remarkable that the incidence of secondary leukemias in patients treated with chemotherapy has not been higher. Today, it is understood that normal cells damaged beyond repair know when to die. Similar damage was presumably also inflicted on the leukemic cells. Nevertheless, 40 years later, many acute leukemias are resistant to treatment with these agents. In this issue of the Journal, Wang et al. (3) provide evidence toward understanding why.

DNA is under constant attack from endogenous toxins, such as free radicals generated by cellular metabolism and exogenous toxins, including many carcinogens. It is, therefore, not surprising that cells have developed multiple mechanisms to ensure DNA integrity. Each DNA repair mechanism corrects a different subset of lesions. DNA double-strand breaks (DSBs) are a genotoxic form of DNA damage because the damage to both DNA strands precludes a straightforward use of the complementary DNA strand as a template in the repair process. There is extensive evidence that DSBs are responsible for chromosomal instability and rearrangements. DSBs arise through the direct action of ionizing radiation or chemicals, including many chemotherapeutic agents. They can also arise when replication of a chromosome that contains a break in one of the two DNA strands converts the single-strand break to a DSB on one of the sister chromatids as the replication fork collapses. Indeed, DNA replication has an intrinsic probability of DSB formation that results in about 10 DSBs per cell cycle, and these DSBs can be efficiently repaired (4).

Human cells can repair DSBs by either homology-directed or nonhomologous repair pathways (5–7). Homologous recombination (HR) repairs DSBs using the undamaged sister chromatid as a template, removing damage in an error-free process. HR is performed by the Rad52 epistasis group of proteins, a large family of proteins variably related to the yeast Rad52 protein. The process of HR involves the following: 1) recognition of the damaged DNA ends, 2) nucleolytic processing of the DNA to produce a single-stranded 3' overhang at each broken end, 3) a search for a homologous template, 4) formation of a “homologous joint molecule” between the 3' overhang on the broken DNA ends and the template, 5) extension of both 3' ends by the synthesis of strands complementary to the templates, and 6) dissociation of the recombination intermediate. The single-stranded DNA that is produced early in this process is recognized by Rad51, the structural and functional homologue of the Escherichia coli RecA protein. Rad51 binds to the single-strand ends, forming a “nucleoprotein filament,” and mediates the search for the homologous template, formation of the joint molecules, and other steps. Other proteins including replication protein A (RPA), Rad52, Rad54, several Rad51-related proteins (Rad51B, Rad51C, Rad51D), x-ray repair cross complementing proteins 2 [Xrcc2], and 3 [Xrcc3]), BRCA1, and BRCA2 function as accessory proteins for Rad51 at various steps.

By contrast with HR, nonhomologous end joining (NHEJ) does not require homology to couple the DNA ends generated in DSBs. Under normal circumstances, NHEJ repairs the DSBs generated during V(D)J immunoglobulin recombination and T-cell receptor gene rearrangements. Because a homologous strand is not required, fewer proteins are involved. These include the Ku heterodimer (Ku70 and Ku86), which has a high affinity for DNA ends and activates DNA-dependent protein kinase (DNA-PKcs) by stabilizing its interaction with the DNA ends, and a complex that consists of DNA ligase IV and Xrcc4, which is involved in the final ligation step. Because NHEJ simply attaches ends together and does not use a template, it is more prone and more likely to result in chromosomal damage.

Because HR requires a template, this mechanism is most efficient in the late S and G2 phases of the cell cycle, when a sister chromatid is available as a template. Acting in coordination with the S and G2 checkpoint controls, HR helps to eliminate chromosomal damage before cell division occurs. By contrast, NHEJ is more likely to be the repair mechanism used during S1 or G1 and on unreplicated DNA in S phase. Furthermore, although HR is used by organisms ranging from yeast to mammals, its importance depends on the organism. Although yeast use HR as the principal pathway for DSB repair, mammalian cells presented with similar substrates appear to favor NHEJ. However, accumulating evidence (8,9) suggests that, in mammalian cells, HR is more important than previously thought. Accumulating evidence (10,11) also indicates that the relative contribution of HR and NHEJ varies, depending on the cell lines used, the phase of the cell cycle, and the stage of development.

Because chemotherapeutic agents in common use, including alkylating agents (cisplatin, carboptatin, and nitrogen mustards, such as melphalan), inhibitors of DNA topoisomerase II (including the anthracyclines, etoposide, and teniposide), and inhibitors of topoisomerase I and antimetabolites are known to or likely to
induce DSBs, understanding several issues related to DSB repair is important for the clinical oncologist.

1) Chromosomal rearrangements are often etiologic in cancer (e.g., bcr-abl and CML), but can normal cells be distinguished from cancer cells by their ability to repair DSBs? Lacking hard data and comparative studies, the answer may be “no.” Although malignant cells may appear to be disordered and unchecked, in reality, cancer cells are very stable units. When first isolated, some cell lines have numerous chromosomal abnormalities, but the majority remain relatively stable over time. Indeed, marker chromosomes are routinely used to “fingerprint” a cell line many years and thousands of cell divisions after the line is originally established. A finite number of DSBs occur in each cell division in both normal and cancer cells, providing opportunities for rearrangements or chromosomal changes to occur. It is, therefore, not surprising that a cancer cell’s chromosomes are abnormal.

Moreover, if a cancer cell’s ability to repair DSBs were indeed impaired, cancer therapies would be vastly more effective. Alkylating agents would result in extensive chromosomal damage and almost certain cellular demise, which is not the case. Thus, the chromosomal abnormalities found in tumors are not likely to be a consequence of a direct failure of DSB repair systems, but rather they reflect accumulated changes in cells with defects in “the coupling of repair with modulation in cell cycle progression” (5).

2) Even if a cancer cell cannot be discriminated from a normal cell on its DNA repair capabilities, can DNA repair be modulated as a therapeutic strategy? Although HR and NHEJ can substitute for each other under certain circumstances, it is not more redundancy. For example, disruption of either HR (Rad51, Rad51B, Rad51D, and Xrcc2) or NHEJ components (DNA ligase IV and Xrcc4) in mice results in embryonic lethality (12–19); rodent cells mutant for any of the genes that participate in NHEJ (ku70, ku86, DNA-PKcs, lig4, or xrc4) show high sensitivity to irradiation, as evidenced by cell death and the acquisition of chromosomal abnormalities (20); and chicken DT40 cells in which one of the genes involved in HR (Rad51b, Rad51c, Rad51d, xrc2, and xrc3) is knocked out all show high sensitivity to cross-linking agents and spontaneous chromosomal abnormalities (21,22). Furthermore, the observation that therapeutic doses of chemotherapy can overwhelm the DNA repair process suggests that the repair system’s capacity is limited.

Mammalian cells, rather than having redundancy in, or an excess capacity for, DNA repair, rely instead on destruction of extensively damaged cells as the backup system. Thus, for example, mice lacking components of the NHEJ pathway (and impaired DSB repair) do not have a high incidence of lymphoid tumors; instead, they have a relatively long latency to tumor development and even the absence of tumorigenesis because of efficient p53-dependent apoptosis of their lymphoid cells (23–25). The crucial role of p53 in apoptosis was demonstrated in mice that, in addition, had a homozygous Trp53 mutation in which tumors developed very rapidly. The importance of NHEJ for genomic stability and suppression of translocations and the role of DSBs in tumorigenesis were revealed by spectral karyotyping analysis, which found translocations involving the T-cell receptor or immunoglobulin heavy-chain loci in the tumors (26), underscoring the importance and the interaction of the NHEJ and p53-mediated apoptosis pathways. In this regard, some investigators (27) have suggested that modulation of the “apoptotic threshold” in cancer cells may be a therapeutic strategy to increase the lethality of DNA damage. Although this approach may work in some instances (e.g., gene therapy to introduce p53), it is unlikely to succeed as a chemotherapy resistance reversal strategy. As Wang et al. (3) and previous investigators (28–30) have shown, resistance to chemotherapy agents, including melphalan, is often mediated by enhanced DNA repair, enhanced drug metabolism, or reduced drug accumulation, mechanisms that all preclude the “apoptotic decision point.”

3) Several investigators have proposed using agents that interfere with DNA repair as a therapeutic strategy. Such agents might directly inhibit either or both DSB repair pathways or abrogate cell cycle checkpoints. The latter is an appealing strategy because it is clear that the success of the DSB repair pathways in maintaining chromosomal integrity is crucially dependent on cell cycle checkpoints. HR, for example, depends on the G2/M checkpoint to allow it time to repair damage acquired during DNA replication or in G2. A failure of the G2/M checkpoint increases the likelihood that cells will enter mitosis with damaged chromosomes. Kinase inhibitors targeting Chk1 and Chk2/hCds1 have been shown to sensitize cancer cells to chemotherapy and have been proposed as adjuvants (31–33). However, this therapeutic strategy has the potential to enhance chemotherapy toxicity and the incidence of secondary malignancies, especially leukemias. For example, in breast cancer patients, secondary leukemias are most likely to occur following the administration of alkylating agents and topoisomerase II inhibitors, the agents that most effectively cause DSBs (34–37). Given that the addition of agents that interfere with DNA repair is in effect a dose intensity strategy (greater damage comparable to that obtained with higher doses), an increase in secondary leukemias seems a real possibility. Furthermore, the therapeutic window for this strategy is unlikely to be favorable, because a majority of cancer cells already have dysfunctional cell cycle checkpoints. Although we have much to learn, it is clear that repair of DSBs is important for both carcinogenesis and cancer therapeutics. Using a wide range of cell lines and thorough evaluating the DSB repair proteins and their activities, Wang et al. (3) have provided a good starting point. Theoretical concerns aside, clinical trials attempting to modulate DNA repair as a therapeutic strategy need to be conducted to determine its value. If we can demonstrate the efficacy of this strategy, we will then have to ensure its safety.

REFERENCES


