Human Topoisomerase II Function, Tyrosine Phosphorylation and Cell Cycle Checkpoints (44240)

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Abstract. **Three DNA damage-responsive cell cycle checkpoints can be shown to operate in diploid human fibroblasts. One checkpoint arrests growth in G1, another inhibits replicon initiation in S phase cells, and the third delays progression from G2 into mitosis. Progression from G2 into M is controlled in part by a cyclin-dependent kinase (cyclin B/Cdk1) that is regulated by tyrosine phosphorylation. Phosphorylation of Tyr15 on Cdk1 is inhibitory for kinase activity. Activation of cyclin B/Cdk1 at the onset of mitosis is accomplished by a phosphatase, Cdc25C, that interacts with cyclin B/Cdk1 in an autocatalytic feedback loop to remove the inhibitory phosphate at Tyr15 and activate kinase activity. DNA damage triggers G2 delay by inhibiting formation of the autocatalytic feedback loop so that dephosphorylation of Tyr15 does not occur. This suppression of activation of cyclin B/Cdk1 appears to account for the failure of damaged G2 cells to progress into mitosis. Once the damage to DNA is repaired, cells resume progression into mitosis as the cycle is re-engaged. The isoflavone genistein inhibits tyrosine kinases, including one that phosphorylates Cdk1 on Tyr15. This kinase, p56/p53^{lyn} is rapidly induced by treatments that trigger cell cycle checkpoints (ionizing radiation, cytosine arabinoside), suggesting that this kinase may actively delay the onset of mitosis by phosphorylating Tyr15 on Cdk1.**

Genistein also inhibits type II DNA topoisomerase to produce a form of DNA damage that triggers all of the DNA damage–responsive cell cycle checkpoints. A brief 10 min incubation with the topoisomerase poison amsacrine was sufficient to trigger the S phase checkpoint response and inhibit replicon initiation. Inhibition of replicon initiation by 1 μ*M* amsacrine was maximal 20–30 min after drug treatment and by 120 min, the checkpoint response had decayed to allow near control rates of replicon initiation. Topoisomerase II poisons also are powerful clastogens inducing lethal and carcinogenic chromosomal aberrations. Type II topoisomerase can break DNA in a region of chromosome 11q23 that contains the ataxia telangiectasia gene (ATM). The ATM gene controls all of the DNA damage–responsive cell cycle checkpoints. Chromosomal aberrations in 11q23 are frequently seen in acute myeloid leukemia that develops as a consequence of etoposide chemotherapy. Thus, topoisomerase poisons such as genistein may trigger chromatid breakage to inactivate AT gene function, disable cell cycle control, and induce genetic instability. [P.S.E.B.M. 1998, Vol 217]

The demonstration that the isoflavone genistein inhibited mammalian type II topoisomerase function and stabilized the topoisomerase-DNA cleaved complex (1) raised the possibility that this compound possesses genotoxic potential. Stabilization of the cleaved complex of calf thymus DNA topoisomerase II on DNA was detected as a drug-induced linearization of closed-circular DNA molecules. In the absence of drug, transient complexes between topoisomerase II and DNA were indetectable. Increasing concentrations of genistein induced increased numbers of linear plasmid molecules indicating the stabilization of topoisomerase II-DNA cleaved complexes. Stabilization of cleaved complexes by topoisomerase poisons is thought to underly their genotoxicity and efficacy as antineoplastic drugs (2). Human and murine cancer cells that were selected for resistance to genistein also display cross-resistance to

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other topoisomerase poisons (3, 4). Thus, cytotoxicity by genistein appears to be attributed to its ability to inhibit topoisomerase II.

Genistein also competes with ATP for binding to protein tyrosine kinases thereby inhibiting tyrosine kinase activity (5). For example genistein inhibits the activity of the p56/p53^{lyn} kinase, which plays a role in signal transduction in lymphocytes (6). The observations that p56/p53^{lyn} may be induced in HL-60 leukemia cells by ionizing radiation (6), that the kinase is associated with the Cdk1 cyclin-dependent kinase, and that p56/p53^{lyn} can phosphorylate Tyr15 in Cdk1 (7), all suggest that the p56/p53^{lyn} tyrosine kinase may negatively regulate cell division. Cyclin B-associated Cdk1 kinase appears to be the final effector molecule in the G2 checkpoint that prevents the movement into mitosis of cells with damaged chromatids. Inhibition of p56/p53^{lyn} by genistein might have the effect to reduce or prevent G2 delay as induced by DNA damage, and thereby enhance the cytotoxicity associated with topoisomerase inhibition.

Etoposide is a chemotherapeutic drug used in treatment of several types of cancer. Etoposide inhibits type II topoisomerase and stabilizes the cleaved complex. Follow-up of patients who have received etoposide therapy revealed increased incidence of acute myelocytic leukemia (AML). Between 2%–12% of patients who receive the epipodophyllotoxins etoposide or teniposide for treatment of one malignancy will develop leukemia as a consequence of this therapy (8). Thus etoposide is a leukemogen. It is also a clastogen inducing DNA double strand breaks and nonhomologous recombination (9). Such DNA lesions are believed to develop in an S-phase-dependent manner as a result of collision between DNA replication forks and drug-stabilized topoisomerase-DNA complexes (2, 9). Such collisions disrupt the cleaved complexes thereby generating DNA double-strand breaks that are not associated with topoisomerase. DNA repair pathways can rejoin double-strand breaks. Join sites typically display losses of sequence information suggesting that the process of repair of double-strand breaks includes some exonucleolytic digestion to create sticky ends that can anneal (10). This processing during repair ensures that even when the broken chromatids are aligned properly some sequence information is lost during rejoining. DNA double-strand breaks also may be rejoined erroneously when broken chromatids are aligned incorrectly thereby generating translocations (11). Erroneous rejoining of double-strand breaks that were generated during immunoglobulin and T-cell receptor rearrangements is thought to underly the activation of *c-myc* and *c-abl* oncogenes in leukemogenesis and lymphomagenesis. Therapy-related AML frequently displays cytogenetic abnormalities at 11q23 including deletions and translocations (8). Sequences of DNA in the 11q23 locus also contain type II topoisomerase cleavage sites (12) suggesting that therapy-related leukemias were induced by topoisomerase-induced DNA breakage in 11q23. It remains to be determined whether serum levels of genistein that may prevent breast or prostate cancer also would induce leukemia.

Cell cycle checkpoints represent positions of control within the cell division cycle that ensure the completion of dependent events and provide more time for repair of DNA damage before DNA replication and mitosis (13). Inactivation of checkpoint function also endows cells with a growth advantage as checkpoint-defective cells continue to proliferate under conditions that normal cells with intact checkpoints arrest growth. Inactivation of checkpoint function by germline or somatic mutations is associated with carcinogenesis, immune deficiency, and neurologic degeneration (14). The most common genetic alteration in cancer, mutation of p53, inactivates a checkpoint that controls the G1/S transition (15). Cells that have lost p53 function display genetic instability and extension of proliferative lifespan (16). Genetic defects in other checkpoint control pathways may also contribute to the genetic instability and enhanced growth that characterizes cancer (14).

Ataxia telangiectasia (AT) is a familial cancer syndrome with 100–300-fold increased incidence of leukemia and lymphoma. Individuals that are heterozygous at the AT locus also display increased risk of breast cancer. The gene that is responsible for the syndrome (ATM) maps to 11q23 and encodes a large protein with homologies to nuclear protein kinases that participate in DNA repair and cell cycle checkpoint responses (17, 18). AT cells display defects in the signalling of DNA damage to cell cycle checkpoints and repair of chromosomal breaks. Thus, mutations in the ATM gene, either inherited or acquired during somatic cell development, could inactivate cell cycle checkpoint function and contribute to carcinogenesis. Is it possible that dietary genistein derived from soy-based food products may penetrate to the nucleus of proliferative stem cells in the bone marrow to inhibit topoisomerase II and p56/p53,^{lyn} thereby both inducing and enhancing chromosomal breakage in 11q23?

In this essay I will present a brief overview of cell cycle checkpoint function in normal (nontransformed) human fibroblasts. I then will discuss in more detail specific features of G1 and G2 checkpoint response and the role of tyrosine phosphorylation in regulation of these checkpoints. Finally I will review what is known of the effect of topoisomerase-induced DNA damage on cell cycle checkpoint function.

Cell Cycle Checkpoints: Dependence Controls and DNA Damage-Response Elements

Genetic studies with yeast, biochemical studies with *Xenopus* egg extracts, and biological studies with mammalian cells all have established that within the eukaryotic cell cycle there are positions where cells may pause pending completion of some essential event (13). These pause sites are known as checkpoints. Checkpoint engagement can be triggered by a variety of stimuli ranging from anaerobiosis, to metabolic imbalance, to DNA damage. Seemingly, cells can sense when conditions are inappropriate for continued progression through the cell cycle and delay further pro-

gression pending correction of the disturbance. Checkpoints may be separated phenomenologically and in some cases genetically into two categories, dependence controls and DNA damage-response elements. Dependence controls monitor the completion of essential events in the cell cycle and can delay subsequent events pending completion of the essential event. Three dependency checkpoints include 1) initiation of DNA synthesis dependent upon completion of mitosis (19), 2) initiation of mitosis dependent upon completion of DNA synthesis (20); and 3) initiation of anaphase dependent upon completion of metaphase (21). In these three cases it is clear that the essential event must be completed before the subsequent event. Failure of dependency checkpoints leads to gross proliferative imbalance. When mitosis is initiated before the completion of DNA replication, cells divide with incomplete genomes. When anaphase is initiated before metaphase is completed, nondisjunction errors produce unequal segregation of chromosomes (aneuploidy). When DNA synthesis is initiated before the completion of mitosis, there is polyploidization or endoreduplication of the genome. These genome-level events appear to characterize cancer cells implying that inactivation or disregulation of dependency checkpoints has severe pathological consequences.

The DNA damage response checkpoints are equally important as they also are frequently disrupted in cancer. Checkpoints have been defined that, in response to a variety of forms of DNA damage, 1) arrest or delay progression from G1 to S, 2) slow the rate of replicon initiation in S phase cells, and 3) delay progression from G2 into mitosis. These three checkpoints can be shown to display differences in their control mechanisms although they all share the requirement for the product of the ATM gene (14, 22). Cells from patients with ataxia telangiectasia display defects in all of the DNA damage-response checkpoints, implying that the ATM gene product has a central role in signaling from sites of DNA damage to elements of regulation of cell cycle progression.

G1 Checkpoint

The G1 checkpoint has been subjected to intense scrutiny in part because it is dependent upon the activity of p53 (15). P53 is a tumor suppressor gene that is mutated in nearly half of all human malignancies, and in large proportions of premalignant precursor lesions (23). As we understand it now, p53 is an essential element of a signaling pathway that appears to begin with the ATM gene product at the site of DNA damage, proceeds through some modification of p53 to increase its half-life and enhance transactivation of p21waf1/cip1/sdi1, which then inhibits G1 cyclin-dependent kinases to block progression from G1 to S phase (24, 25). Damaged cells accumulate in G1 with high levels of cyclin E/Cdk2 complexes that are inactive due to the presence of an excess of p21. G1 arrest in response to ionizing radiation may be quite long-lived with cells remaining blocked in G1 with enhanced expression of p21 for up to 96 hr (26). This observation has suggested that in some diploid normal cells, the G1 checkpoint response to ionizing radiation resembles premature senescence. A variety of forms of DNA damage have been shown to induce p53 to trigger G1 arrest including DNA strand breaks produced directly by ionizing radiation and restriction endonucleases, or indirectly by DNA excision repair, stabilization of topoisomerase-DNA cleaved complexes, or replication of damaged DNA (27, 28). G1 arrest also can be induced by incubating cells in the drug PALA, which inhibits synthesis of nucleotides (16). Inactivation of G1 checkpoint response by mutation in Cdk4 or by inactivation of p53 is associated with increased frequencies of spontaneous or carcinogen-induced mutations and chromosomal aberrations (29, 30). When p53 function is inactivated by human papilloma virus 16 E6, cells acquire genetic instability and develop severe aneuploidization of the genome (16). This genetic destabilization has been linked to a secondary disturbance in G2 checkpoint response (31) indicating that when one cell cycle checkpoint is inactivated to produce genetic instability, other checkpoints undergo progressive failure.

One aspect of G1 checkpoint response to DNA damage deserves further discussion and that is its coupling to DNA excision repair. Nelson and Kastan have reported that xeroderma pigmentosum cells that are unable to repair UV-induced pyrimidine dimers also are unable to induce p53 when DNA replication is inhibited (27). This result implies that pyrimidine dimers in DNA do not directly induce p53 and G1 checkpoint response. However, a recent study by Abrahams *et al.* (32) indicated that some repair-defective XP cells were able to stabilize p53 protein following UV irradiation. UV repair was not quantified in this study so the degree of repair deficit in the XP cells is unclear. Some XP cells can display comparatively high levels of repair of certain forms of UV damage. In normal human fibroblasts, stabilization of p53 was UV-fluence dependent (32). The maximal response occurred after a fluence of 6 J/m^2 that equals the Km for repair endonuclease in the nucleotide excision repair pathway (33). Thus, maximal stabilization of p53 was associated with nearly maximal repair activity in human fibroblasts. Thus, we hypothesize that activation of p53 by UV is triggered by excision repair, and more specifically, by the presence of DNA strand breaks formed during the excision of a 25–27-base oligonucleotide containing the dimer. Once excision repair is complete, with the repair patch having been synthesized and strand breaks rejoined, the signal to induce p53 should subside allowing cells to re-engage progression from G1 to S phase. By coupling the checkpoint response to the repair process, the system is able to determine when sufficient damage has been repaired to risk re-entry into the cell cycle. In our analysis of G1 checkpoint response in human fibroblasts, cells appeared to release into the S phase when UV-induced unscheduled DNA synthesis had subsided by > 75% (34). It is conceivable that repair must diminish to below some thresh-

old level before G1 progression is re-engaged. Recent observations also indicate that induction of p53 by excision repair may further enhance DNA repair capacity (35–37). Several studies have shown that cells lacking wild-type p53 function display reduced repair of UV-induced DNA damage. In one study of Li-Fraumeni cells that expressed only mutant p53, transcription-coupled repair was normal, but global repair was severely retarded (37). The p53-inducible gene GADD45 has been shown to enhance DNA excision repair under specific conditions *in vitro* (35) suggesting that transcription coupled repair is used to induce p53 which then enhances the expression of genes needed to repair damage in less accessible regions of chromatin.

With UV or chemically induced DNA damage, which can be efficiently repaired, a transient delay in progression from G1 to S should be beneficial in reducing the induction of replication-dependent genetic alterations such as base-substitution mutations and chromatid-type chromosomal aberrations. Some forms of DNA damage represent unremitting signals to induce p53 and produce long-lived G1 arrest. Ionizing radiation induces both single and double-strand breaks in DNA. While virtually all single-strand breaks are rejoined by repair, as many as 10% of double-strand breaks may not be rejoined, leaving a continuous signal to arrest growth. These observations suggest that G1 checkpoint response is tied to DNA repair *via* the use of DNA strand breaks as the signal to induce p53. If repair is successful in rejoining all of the breaks, cells may be permitted to continue through the cell cycle. If repair is incomplete and strand breaks persist, cells may arrest growth permanently.

Replicon Initiation Checkpoint

A different checkpoint regulates the rate of replicon initiation in S phase cells. This checkpoint does not require p53 function but does require ATM. The relative resistance of AT cells to radiation-induced inhibition of DNA replication (radiation-resistant DNA synthesis) first pointed to a defect in cell cycle regulation in this disease (38). Subsequent studies showing altered G1 and G2 checkpoint responses in AT confirmed the role of ATM in all DNA damage–response checkpoints (39, 40). The inhibition of replicon initiation is a transient response that is triggered by a variety of forms of DNA damage including ionizing and UV radiations, chemical carcinogens, and topoisomerase poisons (41, 42). The S phase checkpoint response to UV was shown to include rapid inhibition of replicon initiation after low noncytotoxic fluences of UV. Inhibition of replicon initiation was maximal within 30 min after 0.8 J/m^2 and by 120 min after irradiation recovery of initiation had occurred to near control levels. Although DNA excision repair was not required for the inhibition, it was required for the recovery (41). This suggested that pyrimidine dimers themselves or some product of replication of dimer-containing templates (replicative gaps or double-strand breaks) were the signals to induce the response. This checkpoint will be described further below in the discussion of checkpoint response in cells treated with topoisomerase inhibitors. The mechanism of inhibition of replicon initiation has yet to be determined in part because we do not know yet the mechanisms of initiation of DNA replication at replicon origins.

G2 Checkpoint

The G2 checkpoint delays the progression into mitosis of damaged G2 phase cells. When cells in log phase growth are treated with ionizing radiation, there is a dose-dependent reduction in the fraction of mitotic cells, with maximal inhibition of mitosis seen 2 hr after irradiation (31, 39, 43). In diploid human fibroblasts, the D_{37} for mitotic delay is 0.3 Gy which is 20% of the D_0 of 1.5 Gy for cytotoxicity as measured in colony-formation assays. Thus, this checkpoint appears to be quite sensitive to DNA damage and operates with high efficiency after low doses that are in the shoulder region of cell survival curves. After a variable delay that is dependent on the level of DNA damage, cells re-engage the cycle and enter mitosis. The length of G2 delay presumably reflects the time needed to repair DNA to an acceptable level of damage. Within 2 hr after treatment with ionizing radiation, cells will have rejoined 99% of single-strand breaks and 80%–90% of double-strand breaks. After the D_{37} dose of 0.3 Gy, there remains only 1–2 DNA double-strand breaks per cell by 2 hr after irradiation, a time when mitotic inhibition is maximal. By 6 hr after this dose, mitotic activity will recover to or even overshoot control levels (39). AT cells display a quantitative defect in G2 checkpoint response. A dose of radiation that produces 50% inhibition of mitosis in AT fibroblasts will produce 99% inhibition in normal human fibroblasts (22, 31, 39).

Inhibition of mitotic entry during G2 delay has been linked to inhibition of the cyclin-dependent kinase that appears to control the G2/M transition, cyclin B/Cdk1 (31, 44). Cdk1 is the current name for the first cyclin-dependent kinase identified in human cells by virture of its homology to p34^{cdc2} from yeast. Cdk1 can be found associated with cyclins A and B during the cell cycle, but its association with cyclin B best coincides with the G2/M transition (45). Moreover, while cyclin A-associated kinase activity was inhibited by 28% 2 hr after 3 Gy of gamma irradiation, cyclin B-associated Cdk1 was inhibited by 94% (31). Inhibition of cyclin B/Cdk1 therefore was quantitatively associated with G2 delay.

Cyclin B/Cdk1 is regulated by positive and negative phosphorylations (45, 46). Thr161 phosphorylation is required for kinase activity, and this phosphoryl residue appears to be generated by the Cdk-activating kinase, CAK, soon after cyclin B associates with Cdk1. Tyr15 and Thr14 reside within the ATP binding domain of Cdk1 and represent sites of negative regulation on Cdk1. Phosphorylation of these residues inhibits Cdk activity. During G2 as cyclin B/Cdk1 complexes are accumulating, they are inactivated by phosphorylation at these sites by the dual specificity kinase p107^{wee1}. Activation of cyclin B/Cdk1 by removal of the inhibitory phosphates appears to be accomplished by

Cdc25C. This phosphatase is itself activated by phosphorylation suggesting that the formation of a cyclin B/Cdk1/Cdc25C autocatalytic feedback loop (44) may generate an explosive activation of cyclin B/Cdk1 kinase activity to trigger entry into mitosis. In *Xenopus* egg extracts, activation of cyclin B/Cdk1 appears to be sufficient to trigger all the initial events in mitosis including nuclear envelope vesiculation, chromatin condensation, and formation of the mitotic spindle apparatus (45). Cyclin B/Cdk1 also may phosphorylate the motor protein kinesin, which is thought to transport the chromosomes to opposite poles of the spindle during anaphase (45). G2 delay as induced by ionizing radiation, nitrogen mustard, and topoisomerase poisons has been associated with inhibition of cyclin B/Cdk1 kinase and failure to establish the cyclin B/Cdk1/Cdc25C autocatalytic feedback loop (31, 44, 47). Irradiated cells accumulate in G2 with phosphorylated Tyr15. Thus, current investigations are focusing on Tyr15 as a position of control in Cdk1.

The ability of genistein to inhibit tyrosine kinases suggests that some of its effects on cell proliferation may be mediated at this position of control. Tyr15 on Cdk1 and Cdk2 or its equivalent Tyr17 on Cdk4 may represent a key regulatory switch in the basic machinery of the cell cycle (45, 46). The Cdc25 family of phosphatases removes the inhibitory phosphate. Cdc25A appears to function in G1 (48) whereas Cdc25C functions at the G2/M transition (44). Tyr 17 on Cdk4 may play a role in the G1 checkpoint as overexpression of a mutant form of Cdk4, in which Tyr was replaced with Phe, abrogated G1 delay (29). These recent studies suggest that regulation of Tyr phosphorylation on Cdks may be important in cell cycle control.

Normal Functions of Topo II

Mammalian cells express two topoisomerase II genes, α and β (49). Type II topoisomerases catalyze the relaxation of supercoiled DNA and separate intertwined DNA duplexes (decatenation). Relaxation and separation are accomplished by the formation of two protein-linked breaks in DNA where the 5′ phosphoryl group on deoxyribose is covalently joined to a Tyr residue in the topoisomerase (2). The ability of genistein to inhibit both topoisomerase II and tyrosine kinase has been attributed to similar ATP-binding domains in these classes of enzymes (5). Topoisomerase II functions as a homo-dimer with each subunit joining to one of the DNA strands. The unique feature of this complex is that DNA strands may be passed between the two subunits. Thus if the topoisomerase binds to a closed circular duplex, strand passage will relieve supercoiling tension. Topoisomerase II also can separate two intertwined DNA duplexes such as are formed during DNA replication. This function to separate intertwined daughter duplexes in S and G2, appears to be an essential function of topoisomerase II, as yeast mutants that are defective in topoisomerase II function die in G2 (2). Topoisomerase II binding to DNA is sequence-dependent. Sites of cleavage have been mapped within certain mammalian genes (12). Topoisomerase II also is associated with the chromosomal scaffold, and a role in the anchoring of chromatin loops to the nuclear matrix has been suggested (2, 9, 50). The facts that sites of DNA replication and DNA replication forks are associated with the nuclear matrix and that topoisomerase II has an essential function in DNA replication imply that sites of topoisomerase cleavage will be dependent on DNA sequence context, the structural organization of chromatin, and its biological activity (replicating versus nonreplicating).

Topoisomerase II Poisons in Chemotherapy

There are several drugs in the armamentorium of the clinical oncologist that inhibit topoisomerase II function (2). Some of these topoisomerase poisons such as amsacrine intercalate into DNA and prevent the release of covalently bound topoisomerase. In the presence of these drugs one sees protein-associated DNA strand breaks due to stabilization of the cleaved complex. Another class of topoisomerase poisons known as epipodophyllotoxins interferes with the release reaction by binding to the enzyme directly. Etoposide is an example of this class of compounds. Finally a new class of topoisomerase poison has been developed, known as bisdioxopiperazines, that binds to the topoisomerase and prevents formation of the cleaved complex on DNA (51). These compounds presumably kill cells by preventing separation of intertwined daughter duplexes (decatenation).

Stabilization of the cleaved complex on DNA may not be directly cytotoxic. It appears that there must be some secondary event to generate the toxic DNA lesion. One attractive model that has experimental support holds that collision of DNA replication forks with cleaved complexes causes the complex to fall apart without rejoining DNA, thereby generating lethal double-strand breaks (2, 9). Collision of transcription complexes with topoII/DNA complexes might also cause DNA breakage. Incomplete repair of DNA double-strand breaks leads to loss of acentric fragments at mitosis. Erroneous repair may generate dicentric chromosomes that form bridges during anaphase. Breakage of the spindle in such cases causes chromosome losses and gains; breakage of the chromosome generates additional double-strand breaks. The lethal consequences of inhibition of topoisomerase II function are manifest in the high levels of chromosomal aberrations that are induced in drug-treated cells.

Checkpoints Respond to Topoisomerase-Induced DNA Damage

Topoisomerase poisons damage DNA and trigger checkpoint responses. Etoposide can induce p53 in murine and human cells (27, 28). Etoposide also induces the S phase checkpoint response of inhibition of replicon initiation (52). A brief 10 min exposure to the drug was sufficient to trigger the response. Similarly a brief treatment with 1 μ*M* amsacrine induces the inhibition of replicon initiation in diploid human fibroblasts (Fig. 1 (42)). This inhibition is maximal 30–60 min after treatment. By 120 min after treat-

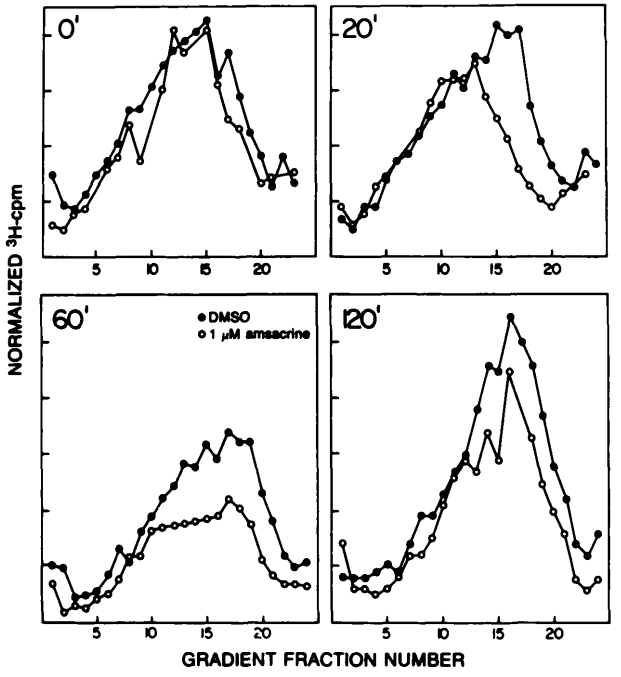

Figure 1. Topoisomerase II-induced DNA damage triggers the replicon initiation checkpoint in S phase human fibroblasts. Normal human fibroblasts in logarithmic growth phase were treated with 1 μ*M* amsacrine for 10 min. At various times after treatment, cells were incubated for 15 min with ^{3}H-thymidine to label newly synthesized DNA. Cells were harvested, lysed on top of alkaline sucrose gradients, and nascent DNA strands separated by velocity sedimentation. After fractionation of gradients, acid-insoluble ^{3}H radioactivity was normalized to the number of cells applied to gradients (42). Reprinted from Ref. 42 with permission.

ment with 1 μ*M* amsacrine, recovery of replicon initiation rate is evident. Etoposide also triggers G2 delay (47). The inhibition of Cdk1 kinase activity is maximal within 30 min after drug treatment implying that the topoisomerase II/DNA cleaved complexes may trigger the G2 checkpoint response directly without the requirement for DNA synthesis (47). As previously mentioned recent studies have focused on p56/p53^{lyn} as a potential effector of G2 delay (6, 7). Inhibition of this tyrosine kinase might have the effect of inactivating the checkpoint so that damaged cells enter mitosis without delay. The methylated xanthine, caffeine, is also known to inactivate all DNA-damage-responsive checkpoints and induces synergistic lethality in combination with chemotherapeutic drugs (53). Treatment of human fibroblasts with amsacrine and caffeine induced extraordinary chromosome damage as seen in mitotic cells (Fig. 2).

Summary

The full genotoxic potential of genistein *in vivo* remains to be established. It is clear that the compound can kill cancer cells. Limited data imply that it may be less damaging to normal human cells, although 24-hr exposure to 10 μ*M* genistein did induce G2 delay in human lymphocytes (54). While DNA double-strand breaks as induced by topoisomerase II poisons are lethal lesions, a subfraction of damaged cells will survive with permanent genetic alterations. If the permanent alterations are in oncogenes or tumor suppressor genes, malignant transformation may be a delayed consequence of therapy. The carcinogenic consequence of etoposide chemotherapy is well documented in the case of AML. Studies to determine the chemopreventive effects of isoflavones also must consider the possibility of adverse reactions initiated by genetic toxicity.

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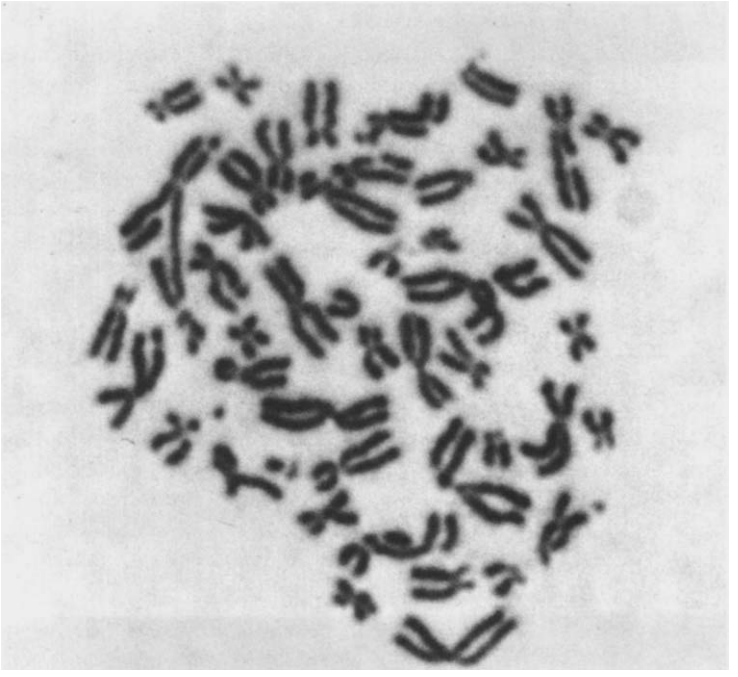

Figure 2. Topoisomerase II-induced chromosomal damage in normal human fibroblasts. Fibroblasts in log-phase growth were treated with 1 μ*M* amsacrine for 10 min, then incubated with 2 m*M* caffeine before harvesting for cytogenetic analysis. Metaphase spreads were stained with Giemsa.

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