

Long-distance inflammatory and genotoxic impact of cancer in vivo

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The relationship between cancer and DNA damage is intimate and multifaceted. Damage to DNA can cause cancer, major treatment modalities including radiation therapy and chemotherapy can cure or slow down cancer through their genotoxic effects, and germline and somatic defects in the DNA damage response (DDR) machinery may predispose to or promote tumorigenesis, respectively (1, 2). Furthermore, replication stress with the ensuing endogenous DNA damage elicited by oncogenes in nascent tumor cells leads to activation of DNA damage checkpoints and cellular senescence or cell death, thereby providing an intrinsic biological barrier against tumor progression (3–5). The PNAS article by Redon et al. (6) adds yet another dimension to the interplay between DNA damage and cancer: a surprising discovery that cancer can elicit long-range signals that evoke serious types of DNA damage in otherwise healthy tissues in various parts of the body distant to sites of the growing tumor.

Conceptually, the new results of Redon et al. (6) are broadly reminiscent of the biological phenomena known as bystander effects (BEs). BEs are best defined in radiation biology, when cells not themselves directly exposed to ionizing radiation show increased genomic instability and impaired viability as a result of effects of substances released by the directly irradiated cells located in the vicinity (7). Similar BEs are known to occur near cancer cells and senescent cells, again causing DNA damage in the nearby bystander normal cells (8–11). What is particularly novel and unexpected in the new PNAS study (6) is the fact that the DNA-damaging effects are observed in distant tissues, rather than limited to cells that share the same local tissue environment with the implanted tumors. Additional valuable features of the new study (6) include the focus on in vivo experiments (rather than cell culture models commonly used in studies on BE), mechanistic insights into the nature of the signaling molecules involved, and the fact that the studied tumors had not been exposed to stresses such as radiation.

What are the major findings reported by Redon et al. (6)? In brief, the authors used two strains of mice, BALB/c and

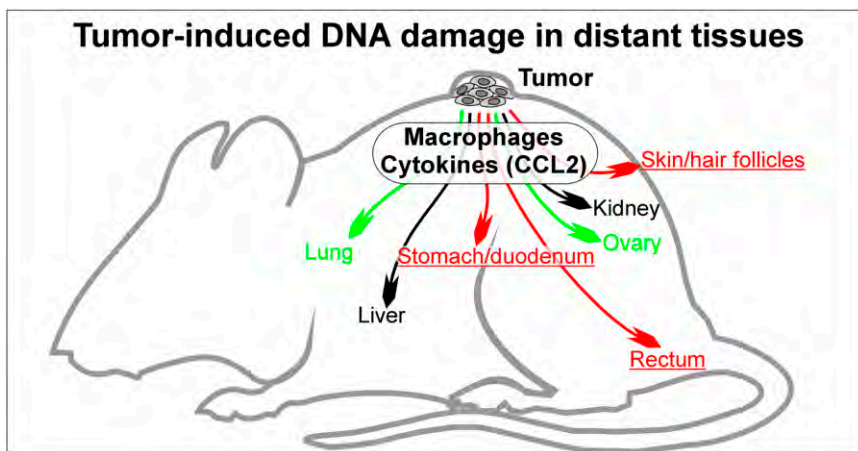


Fig. 1. Induction of DNA damage in distant tissues of tumor-bearing mice correlates with proliferative state of the tissue and depends on the cytokine CCL2, likely secreted by infiltrating macrophages (6). Whereas both OCDLs and DSB lesions occur only in highly proliferative tissues (red), less proliferative tissues harbor OCDLs (but not DSBs) when active macrophages are present (green). Kidney and liver (black) lack either DNA lesions, consistent with the lack of infiltrating macrophages in kidney, and enhanced antioxidant defense in the liver. These results support a scenario whereby macrophages activated in the tumor become recruited to distant tissues and through secreted cytokines including CCL2 evoke enhanced oxidative stress that may lead to DNA breakage during S phase (as detailed in the text).

C57BL/6, to implant s.c. three types of mouse tumors: melanoma, sarcoma, and intestinal carcinoma. When the tumor mass reached 200 mg, the mice were killed, and a wide range of tissues were examined for markers of potentially enhanced DNA damage (Fig. 1). Indeed, the tumor-bearing mice showed increased incidence of two types of serious DNA lesions—DNA double strand breaks (DSBs) and oxidatively induced non-DSB clustered DNA lesions (OCDLs)—in multiple tissues even distant from the sites of tumor growth, such as organs along the gastrointestinal tract (GIT). Intriguing differences in the type of DNA lesions and their frequency were observed in a tissue-dependent manner (Fig. 1). In addition, the authors also found elevated numbers of activated macrophages in the affected tissues and identified several cytokines selectively enhanced in the tumor-bearing animals. Among the tissues examined, all tissues except for liver and kidney showed enhanced levels of OCDLs, whereas only those tissues that are rapidly proliferating, including epithelia of the skin, hair follicles, stomach, duodenum, and rectum, showed elevated numbers of DSBs (6). These results strongly support the notion

that during DNA replication, the cell cycle phase particularly susceptible to DNA damaging insults (5, 12, 13), the cytokine-induced oxidative lesions may become converted into DSBs through collisions of non-DSB lesions such as OCDLs with replication forks and replication intermediates that require complex processing to avoid conversion into mutagenic lesions (12). In this context, it would be extremely interesting to find out whether the observed DSBs occur preferentially in the genomic regions particularly susceptible to replication stress, such as the so-called fragile sites and telomeres (5, 12, 13), which appear to be targeted by the oncogene-associated DNA damage (3–5, 13).

Previous reports implicated diverse inflammatory cytokines as molecules responsible for the BE in various biological settings (7–10). Inspired by these studies, Redon et al. (6) found 4 of 60 examined

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cytokines clearly elevated, hence representing potential candidates for messengers of the long-range DNA-damaging effects observed in the tumor-bearing mice. The authors focused on CCL2 (also known as monocyte chemoattractant protein-1) because of known links of this cytokine with chronic inflammation conditions and cancer (14). When genetically CCL2-deficient mice were implanted with the same tumors, no measurable CCL2 was detected in the sera, and no increase of either DSBs or OCLDs was observed in the GIT tissues that showed maximum DNA damage in the CCL2-proficient animals (6). These exciting results identified CCL2 as an essential mediator of the long-range genotoxic effects and strongly indicated that this critical cytokine is unlikely to be produced by the cancer cells themselves. Indeed, analysis of diverse classes of immune cells identified abundant infiltration of not only the tumors but also distant tissues, by activated host macrophages, a likely source of the elevated cytokines and therefore the key to the DNA-damaging effects (Fig. 1).

Apart from the thought-provoking data itself, the new study raises a host of burning questions. For example, is CCL2 not only required, but also sufficient, or does induction of the observed effects require additional cytokines or even some additional factors? Could these relatively modest increases of DNA lesions indeed promote secondary tumorigenesis in proliferating tissues, as speculated by the authors? This is an important issue, as BEs can indeed be tumorigenic (15), and re-

cent work showed that, in addition, toxins produced by pathogenic bacteria can induce senescence in human cells, including secretion of many cytokines and induction of DNA damage and genomic instability (16). Although not addressed by Redon et al. (6), it would be important to examine

Cancer can elicit long-range signals that evoke serious types of DNA damage.

whether the observed enhanced DNA damage also occurs in stem cells, as the rapidly proliferating transit-amplifying cells are replaced and eventually shed by both skin and GIT tissues, thereby unlikely to give rise to tumors even if their DNA is damaged.

Another key question is to what extent these studies in mice are applicable to human patients. As the authors analyzed the mice when the tumors reached approximately 1% of the host body weight, this would correspond to extremely large tumors—approximately 0.5 to 1 kg—in humans, a scenario that is extremely rare. Mouse cells are also more sensitive to oxidative stress than human cells, and to directly address this question by quantitative analyses of DNA lesions in human tissues will be quite challenging, given the obvious ethical hurdles. Last but not least, it would be exciting to decipher the

entire circle of mutual effects between DNA damage, cancer cells, and the cytokine network. In early lesions, oncogenic stress evokes DNA damage in cancer cells (3–5) and secretion of cytokines that is dependent on the signaling from the DNA damage kinases ATR and ATM (9, 10, 17, 18). It remains to be elucidated how these early events and the local tumor environment are connected to activation of macrophages and thereby to the secondary cycle of cytokine secretion and ensuing DNA damage in distant tissues.

From a broader perspective, the DDR machinery (19) and the inflammatory responses (20) are at the heart of tissue homeostasis and critically involved in cancer development. Arguably, the major contribution of the new study (6) is the demonstration that tumors may induce chronic inflammatory response *in vivo* and undermine the maintenance of genome integrity at a systemic level. This message will no doubt inspire a flurry of new studies to answer the open questions and may eventually allow smart interventions into the complex interplay between the DDR and inflammatory machineries to help cure or even prevent cancer.

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