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Para-inflammation mediates systemic DNA damage in response to tumor growth

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The radiation induced bystander effect is a well-accepted consequence of ionizing radiation exposure. However, it has become clear that bystander responses in vitro can result from a number of stress stimuli. We had reported that media conditioned on tumor cell cultures induced a bystander effect in recipient normal cell cultures and asked whether an analogous process could occur in vivo—could the presence of a tumor induce DNA damage in distant tissues. We recently demonstrated the presence of a distant bystander DNA damage response in vivo in the gastrointestinal organs and skin of mice implanted with subcutaneous tumors. The activation of inflammatory macrophages through the cytokine CCL2 was found to be required for this distant genotoxic response. These results shed new light on the consequences of tumor growth to distant parts of the body and highlight the potential for possible medical interventions to mitigate the effect of cancers.

Key words: MCP1/CCL2, bystander effect, tumor, inflammation, oxidative stress, γ-H2AX

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Cells exposed to ionizing radiation affect their otherwise uninjured neighboring cells. These cells, known as bystander cells, exhibit a variety of genome destabilizing effects which include DNA damage formation, apoptosis, micronucleus formation, senescence, mutations, etc.[1,2] While these bystander effects are similar to those exhibited by the irradiated cells, they differ in timing and extent from the direct radiation-induced effects. The radiation-induced bystander effects have been demonstrated not only in vitro using targeted irradiation, mixed cell cultures and media transfer, but also in vivo as an abscopal effect, where irradiation of one organ results in the response of a distant unirradiated organ.[3,4]

We had put forward the hypothesis that the radiation-induced bystander effect is a specific instance of a more general phenomenon; a stress-induced systemic process. Using phosphorylated H2AX (γ-H2AX) foci formation as a sensitive indicator of DNA double-strand breaks,[5] we demonstrated that a variety of stress factors, non-ionizing radiation, skin irritation agents, wound formation and media conditioned on otherwise untreated senescent and tumor cells, also led to increased DNA damage levels in unstressed cells, similar to the levels reported in ionizing radiation-induced bystander DNA damage.[6]

Our observation that media conditioned on untreated tumor cells is able to induce a DNA damage response in normal cells in culture led us to examine whether an analogous process occurred in vivo. We implanted C57BL/6 and BALB/c mice with three types of subcutaneous syngeneic tumors. When the growing tumors were still non-metastatic, tissues from different parts of the mice were harvested and analyzed for two serious types of DNA lesions—double-strand breaks (DSBs) and oxidative clustered DNA lesions (OCDLs).

Elevated levels of both types DNA lesions were found in tissues throughout the bodies of the mice in all mouse-tumor combinations, but with some significant differences. γ-H2AX foci were elevated in hair follicles, in skin samples taken 0.5 to 2 cm from the tumor mass and

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in gastro-intestinal tract (GIT) tissues.[7] These tissues exhibit rates of cell proliferation among the highest in the body, contain stem and transit-amplifying cells, and are known oxidative stress targets. These results relating bystander DNA DSB induction with replicative status of the tissues are in agreement with previous reports pointing to S-phase cells as the most vulnerable targets for bystander signaling.[8,9]

The other type of DNA damage examined, OCDLs, also exhibited elevated levels in these same tissues. In addition, they were elevated in other tissues that lacked elevated levels of γ-H2AX foci, ovary and lung. OCDLs form independently of cellular proliferative status and thus may be more widespread than γ-H2AX foci.

We set out to determine the nature of the agent inducing the distant DNA damage. In vitro studies have implicated a variety of molecules and factors in the bystander effect.[9] These include reactive oxygen and nitrogen species (ROS and NOS), a variety of cytokines and members of the COX-2 pathway. Additionally, treatment of cells in culture with TGF-β, a protein involved in many cellular processes including the inflammation response, resulted in γ-H2AX foci formation.[10] Of these candidates, ROS and NOS are generally considered to be short-lived and perhaps not likely to survive transport from tumor to distant tissue. However, cytokines are stable and present in serum. Thus, we compared the cytokine/chemokine profile of serum from tumor-bearing mice to control serum to identify candidate agents that may play causal roles in tumor-induced distant DNA damage. Of 64 proteins examined, the plasma levels of four were substantially elevated, CCL2, CCL4, CCL7 and CXCL10. These factors are involved in activating and attracting monocytes to sites of tissue damage or activating tissue-resident macrophages.[11] To examine whether CCL2 played a causal role in tumor-induced distant DNA damage, we compared tumor-bearing CCL2-null and wild-type mice. Strikingly, no elevation of DNA damage levels was found in GIT tissues of the tumor-bearing CCL2-null mice, indicating that CCL2 plays an essential role in this process. CCL2 is secreted by many cell types including tumor, normal and immune cells and has been implicated in the pathogenesis of diseases characterized by monocytic infiltrates, like psoriasis, rheumatoid arthritis and atherosclerosis.[12-14]

The finding that CCL2 is essential for the induction of tumor-induced distant DNA damage led us to examine the various tissues exhibiting or not exhibiting damage for the presence of immune cells. Cancers have been shown to induce a persistent inflammatory response in the animal, leading to the establishment of an inflamed microenvironment which may be infiltrated by a variety of immune cells.[15] One species of commonly recruited immune cells is the growth promoting tumor-associated macrophage (TAM).[16] TAMs consist primarily of a polarized M2 ($F4/80^+/CD206^+$) macrophage population with little cytotoxicity for tumor cells because of their limited production of NO and proinflammatory cytokines.[17,18] Importantly, GIT tissues and skin of tumor-bearing mice exhibited greater numbers of $F4/80^+$ macrophages compared to controls. Also as expected, the implanted tumors harbored large populations of $F4/80^+$ macrophages.

The observations reported here suggest a model for the induction of systemic DNA damage in the presence of tumor growth (**Fig. 1A**). OCDLs are signature oxidative DNA lesions, so we hypothesize that ROS are the immediate damaging agent. The variations come from whether the ROS are generated by tumor cells or by activated macrophages, and whether the activated macrophages present in the distant tissues were resident and activated in situ or whether they were activated elsewhere, such as in the tumor, and migrated to the distant tissues. We hypothesize that CCL2 is necessary for the macrophage activation. The ROS would damage the genome through the induction of oxidative DNA lesions (abasic sites, base lesions and/or single strand breaks resulting in the formation of OCDLs). In highly proliferative tissues, the replication machinery significantly increases the risk of DSB induction when replication forks collide with a damaged DNA template (**Fig. 1B**).

Thus, one important conclusion from this work is that while distant tissues exhibit a bystander-like effect in vivo, it should be distinguished from the bystander effect in vitro due to the involvement of the immune system. The involvement of CCL2 and macrophage activation in tumor-induced DNA damage suggests similarities with the chronic tissue stress responses, named para-inflammation,[11] which relies principally on alternatively activated macrophages (M2) in response to a chronic condition rather than on classically activated macrophages (M1) associated with an acute inflammatory response.[19] We hypothesize that the increase of F4/80-positive macrophage numbers throughout the body of the animal induced by the chronic tumor-induced inflammation would result in an altered homeostatic state leading to genotoxic stress throughout the body. These observations show that the presence of a tumor may have a much more widespread effect the body. While this is accepted in later-stage metastatic cancer, our results indicate that even very early stage non-metastasized tumor growth may have profound effects on the body and inflammatory response pathway. These results may give insight on the role of inflammation on cancer risk and progression, and hopefully lead to possible medical interventions to mitigate the effect of cancers on the body.

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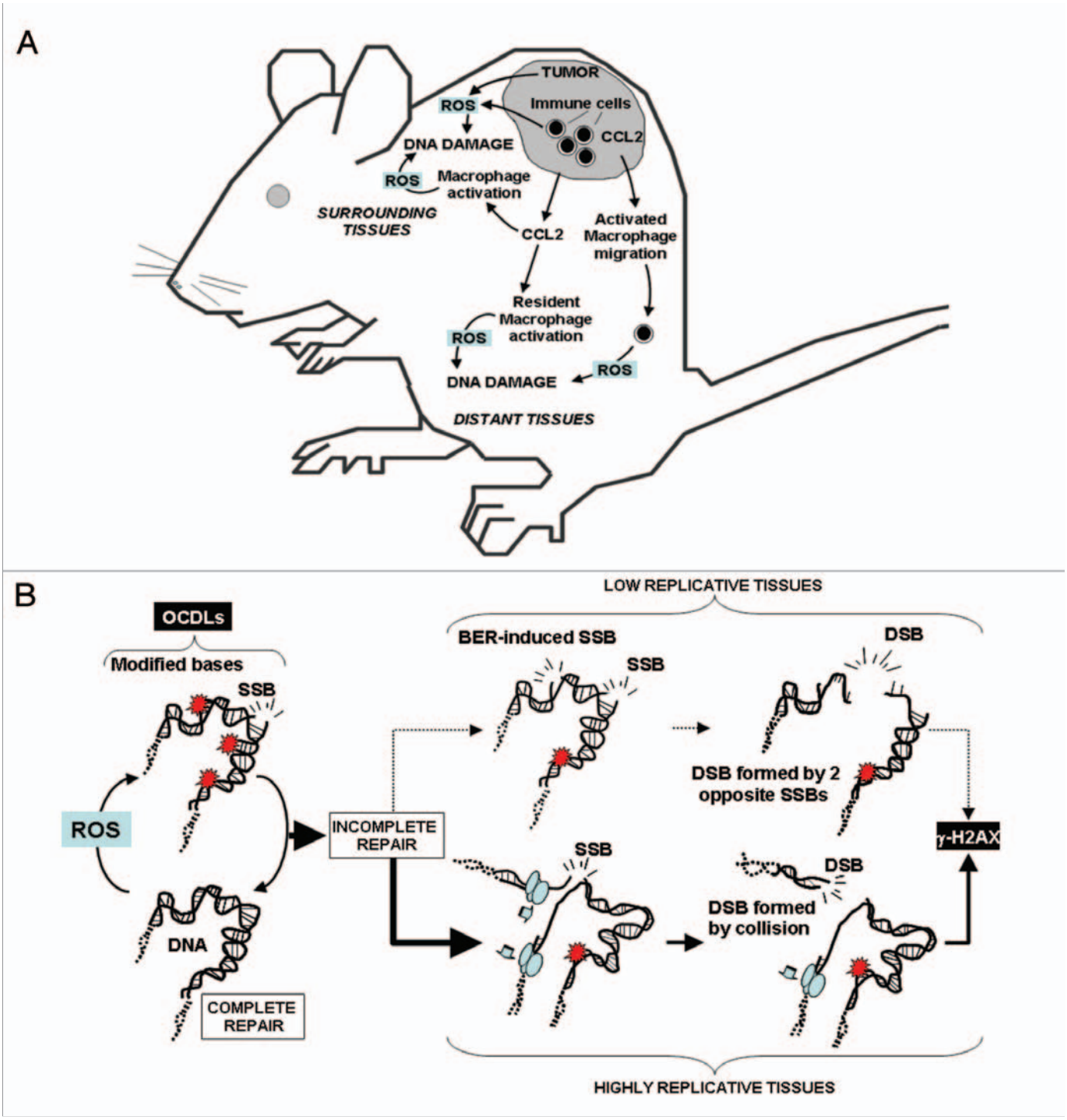

Figure 1. Tumor-induced DSB formation in vivo. (A) Tumors could promote DSB formation in normal tissues in two ways. First, through persistent ROS release which directly target DNA of surrounding tissues. Second, tumor cells generate an immune response leading to cytokine production, including CCL2, by tumor-infiltrated immune cells. CCL2 then targets both surrounding and distant normal tissues and recruits and/or activates macrophages which, in turn, induce the production of ROS in those tissues. However whether the activated macrophages present in the distant tissues were resident and activated in situ or whether they were activated elsewhere, such as in the tumor, and migrate to the distant tissues is still to be determined. (B) ROS affect normal adjacent and distant tissues through the induction of abasic sites, base lesions (red spots) and/or single strand breaks (SSBs) resulting in the formation of OCDLs. While isolated damages are generally repaired efficiently, OCDLs are more difficult to repair, and DSBs in both replicating and non-replicating cells can be formed indirectly by the base excision repair (BER) pathway when a BER-induced SSB happens to be generated opposite a pre-existing SSB, or two nearby clustered lesions on complementary DNA strands can produce a DSB. In highly proliferating tissues, the replication machinery significantly increases the risk of DSB induction as accumulating oxidative lesions can result in DSBs formation due to the collision of replication forks with a damaged DNA template.

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