Identifying the Stroma as a Critical Player in Radiation-Induced Mammary Tumor Development

Magdalena A. Cichon,[1] Evette S. Radisky,[1] and Derek C. Radisky[1,*]
[1]Department of Cancer Biology, Mayo Clinic Cancer Center, Jacksonville, FL 32225, USA
*Correspondence: radisky.derek@mayo.edu
DOI 10.1016/j.ccr.2011.05.001

Tumor development requires facilitative alterations in the stroma. In this issue of *Cancer Cell*, Nguyen et al. provide evidence that irradiation of mammary stroma drives accelerated development of mammary tumors that are more likely to be estrogen receptor-negative and implicate stromal production of transforming growth factor-β in this process.

Many epidemiological studies have revealed an increased risk of subsequent cancer development in individuals exposed to ionizing radiation (IR). As radiation is well known to cause DNA damage both directly and indirectly through induction of reactive oxygen species (ROS), most investigations of IR as a carcinogen have focused on how incorrect or inefficient repair of IR-induced DNA damage could potentially lead to cancer-associated mutations. However, even strongly activating oncogenic mutations are not sufficient for tumor formation without facilitative changes in the microenvironment of the tumor cells (Bissell and Radisky, 2001), changes, which can be caused by IR (Barcellos-Hoff, 2010). Persistent alterations induced by IR in the stroma surrounding the developing cancer are known as "bystander" or "nontargeted effects," and it is clear that these can be induced by levels of radiation far below those necessary to induce widespread DNA damage (Barcellos-Hoff et al., 2005; Wright, 2010).

Epidemiological studies have found that women exposed to IR during mammary gland development have an increased risk of early onset cancers that are more likely to be estrogen receptor-negative (Castiglioni et al., 2007). The study reported by Nguyen et al. (2011) in the current issue of *Cancer Cell* uses an elegant experimental model to show how both of these characteristics could be caused by the effects of IR on the stroma, independent of any direct effect of IR on the epithelium (Figure 1). Differentiating the contribution of stromal and epithelial tissue in the development of mammary gland tumors can be performed by transplanting mammary gland tissue into cleared mammary fat pads of isogenic mice. Transplanted tissue deficient for expression of the tumor suppressor *Trp53* will eventually develop tumors with many characteristics of human breast cancer, including heterogeneous histology and differential expression of estrogen receptor. Nguyen et al. (2011) show that irradiation of mice prior to implantation leads to significantly shorter tumor latency, and this effect can be observed even following exposure to low levels of IR; doses as low as 10 centigrays (cGy) are found to decrease tumor latency. Nguyen et al. (2011) further show that this effect is dependent upon stromal production of transforming growth factor-β (TGFβ), as irradiation of $Tgf\beta^{+/-}$ mice prior to transplantation did not significantly decrease tumor latency. Strikingly, a greater proportion of the tumors that developed in the irradiated mice were found to be deficient for expression of estrogen receptor-α, although this effect appeared to be independent of TGFβ, as irradiation increased the proportion of ERα-negative tumors similarly in wild-type and $Tgf\beta^{+/-}$ mice.

How does stromal irradiation stimulate the development of nonirradiated epithelial tissue? Identification of the mechanism(s) could potentially lead to interventions that benefit individuals exposed to radiation, reducing subsequent cancer incidence or severity. Nguyen et al. (2011) implicate stromal production of TGFβ is as a component of IR-induced tumor development. Previous studies from the Barcellos-Hoff group identified an IR-induced, ROS-dependent mechanism by which latent TGFβ is converted into active TGFβ in irradiated mammary glands (Barcellos-Hoff et al., 1994), finding substantial and persistent activation even in mice given radiation doses as low as 10 cGy (Ehrhart et al., 1997). IR-induced activation of TGFβ can induce fibrosis-like conditions, including collagen deposition, which can disrupt tissue structure and facilitate tumor invasion (Radisky et al., 2007). Another mechanism by which irradiated stroma may stimulate tumor development is through effects on immune cells, which are known to play critical roles in breast cancer development. This could also occur through TGFβ, which is known to inhibit an endogenous anticancer gene program in cytotoxic T cells (Thomas and Massague, 2005). Alternatively, irradiation of C57Bl/6 mice was shown to stimulate an increase in activated M2 macrophages (Coates et al., 2008), immune cells that have been linked to increased tumorigenesis in experimental models and that are associated with a poor prognosis in breast cancer patients.

The study reported by Nguyen et al. (2011) strikingly illustrates the significance of both global environmental exposures and consequent microenvironmental influences in determining whether susceptible premalignant epithelial cells escape latency and go on to become malignant. At the same time, this study brings into focus many more unanswered questions, not only with regard to the molecular mechanisms alluded to above, but also relating to the risks associated with human radiation exposures. The lowest dose used in this study, 10 cGy, is comparable to typical mission doses on the International Space Station (Barcellos-Hoff et al., 2005) and is in the range of many medical procedures. In light of the finding that even relatively low doses of stromal irradiation can significantly affect later cancer development from mutant epithelial cells, it will be

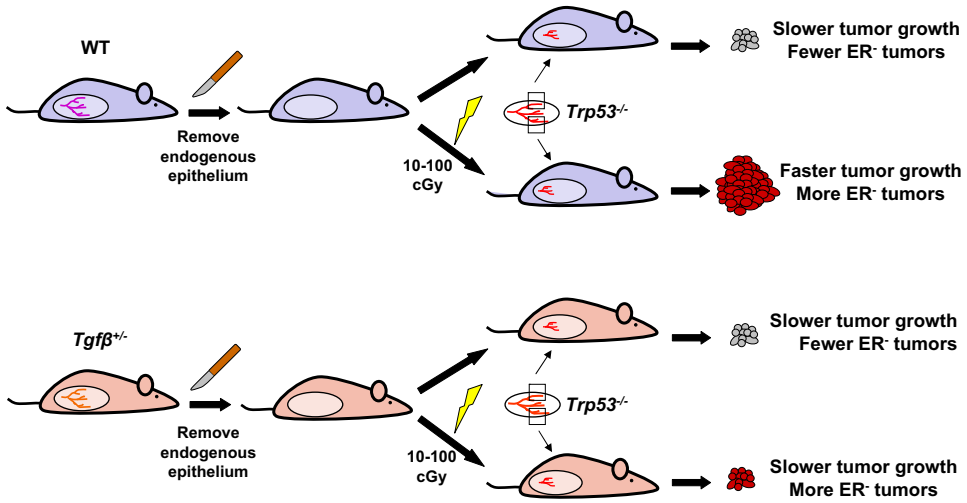

Figure 1. Experimental Model to Define Radiation-Induced Stromal Effects on Tumor Development

In 3 week old mice, the rudimentary mammary epithelium can be completely removed through a surgical procedure known as fat pad clearance. Subsequent allografting of mammary gland tissue into the cleared fat pads can lead to mammary ductal outgrowths and, if the transplanted epithelium is from a *Trp53*-deficient mouse (*Trp53*$^{-/-}$), eventual tumor development. When epithelium-cleared mice were irradiated with a dose of 10–100 cGy prior to allograft, tumors developed more quickly, and an increased proportion of the tumors were estrogen receptor-negative (ER^{-}). The radiation-induced reduction in tumor latency was dependent upon stromal activation of TGFβ, as tumor outgrowths from irradiated *Tgfβ*$^{+/-}$ mice did not develop significantly faster than in nonirradiated mice. By contrast, irradiation increased the proportion of ER^{-} tumors in mice of both genotypes, suggesting that this propensity may be independent of TGFβ.

important to better define the relationship between thresholds of radiation exposure and later cancer risk. It also is important to better understand how the temporal juxtaposition of radiation exposure and mammary gland development contribute to subsequent cancer risk. A thorough understanding of these risk factors and a more complete understanding of the molecular mechanisms by which radiation promotes later breast carcinogenesis is necessary to better define effective interventions to prevent or reduce the incidence of radiation-associated breast cancer.

REFERENCES

Barcellos-Hoff, M.H. (2010). J. Mammary Gland Biol. Neoplasia *15*, 381–387.

Barcellos-Hoff, M.H., Derynck, R., Tsang, M.L., and Weatherbee, J.A. (1994). J. Clin. Invest. *93*, 892–899.

Barcellos-Hoff, M.H., Park, C., and Wright, E.G. (2005). Nat. Rev. Cancer *5*, 867–875.

Bissell, M.J., and Radisky, D. (2001). Nat. Rev. Cancer *1*, 46–54.

Castiglioni, F., Terenziani, M., Carcangiu, M.L., Miliano, R., Aiello, P., Bertola, L., Triulzi, T., Gasparini, P., Camerini, T., Sozzi, G., et al. (2007). Clin. Cancer Res. *13*, 46–51.

Coates, P.J., Rundle, J.K., Lorimore, S.A., and Wright, E.G. (2008). Cancer Res. *68*, 450–456.

Ehrhart, E.J., Segarini, P., Tsang, M.L., Carroll, A.G., and Barcellos-Hoff, M.H. (1997). FASEB J. *11*, 991–1002.

Nguyen, D.H., Oketch-Rabah, H.A., Illa-Bochaca, I., Geyer, F.C., Reis-Filho, J.S., Mao, J.H., Ravani, S.A., Zavadil, J., Borowsky, A.D., Jerry, D.J., et al. (2011). Cancer Cell *19*, this issue, 640–651.

Radisky, D.C., Kenny, P.A., and Bissell, M.J. (2007). J. Cell. Biochem. *101*, 830–839.

Thomas, D.A., and Massague, J. (2005). Cancer Cell *8*, 369–380.

Wright, E.G. (2010). Mutat. Res. *687*, 28–33.