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Response to the Letter by Colin Seymour and Carmel Mothersill

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The interesting comments of Seymour and Mothersill emphasize the varied phenomena associated with effects in “bystander” cells in irradiated populations. In their experiments (1), conditioned medium from immortalized epithelial cells exposed to γ radiation was found to have a cytotoxic effect when added to nonirradiated cells growing on another dish (these recipient cells were labeled bystander cells). The addition of TPA to the growth medium of the donor cells prior to irradiation enhanced the cytotoxic effect in the recipient cells rather than attenuating it (1). Owing to the lack of attenuation of the cytotoxic effect, it was concluded that communication of the damage signal in donor cells via gap junctions does not contribute to the bystander response in the recipients. As pointed out in their letter, a role for intercellular communication was observed in a different study (2).

The experimental protocol adopted in our studies (3) differs significantly from that employed by Mothersill and Seymour. We exposed confluent density-inhibited normal human diploid fibroblast cultures to very low fluences of α particles where only a small fraction of the cells’ nuclei were traversed by an α -particle track (3). We measured the end point of gene expression rather than clonogenic survival. We observed an induction of the TP53/CDKN1A signaling pathway in a significantly greater fraction of cells than those whose nuclei were traversed by an α -particle track. In the presence of the gap-junction inhibitor lindane (at concentrations which did not alter survival after exposure to α particles or γ rays), induction of TP53 and CDKN1A was reduced in bystander cells. Such a protocol where hit and nonhit cells coexist in the same culture dish is easily achievable in studies using α particles. At the lowest dose of ^{60}Co γ rays (0.5 Gy) used in the studies of Mothersill and Seymour (1, 2), every cell in the population would be exposed to multiple photons (4), thus resulting in the absence of nontraversed cells in the population. As a result, inhibition of gap-junction-mediated intercellular communication as described in ref. (1) might well have no effect, as every cell in the population received direct radiation exposure and could potentially secrete the diffusible cytotoxic factor.

The fact that a released factor effect was not observed by Mothersill and Seymour when medium from irradiated immortalized fibroblasts as opposed to epithelial cells was harvested and added to nonirradiated cells (1, 5) emphasizes the contribution of other effects to the bystander response observed in normal human fibroblasts (3, 6). As originally postulated by Nagasawa and Little (7), recent studies by Narayanan *et al.* (6) and unpublished results from our laboratory indicate that reactive oxygen species may also be mediators of the bystander response. However, we do not discount that a factor may also be released in the medium of α -particle-irradiated cells that can modulate gene expression in bystander cells. We are currently exploring this possibility.

The studies of Mothersill and Seymour have provided intriguing results in exploring the importance of bystander effects in the cytotoxic response of cells to low-LET radiation. In terms of intercellular communication, it is unfortunate that inhibitors such as TPA and lindane are rarely specific in their effects. The use of cell strains that are proficient in intercellular communication and of isogenic strains deficient in this process would eliminate the need for their use. The availability of such strains as well as knockout cells and microbeam irradiators should soon help to increase our understanding of the molecular pathways underlying these untargeted responses.

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