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Letters to the Editor

Correspondence re: S. Fulda *et al.*, Betulinic Acid Triggers CD95 (Apo1/Fas)- and p53-independent Apoptosis via Activation of Caspases in Neuroectodermal Tumors. Cancer Res., *57:* 4956–4964, 1997.

Letter

In their recent article, Drs. Fulda *et al.* (1) propose that BA[1] a natural pentacyclic terpene, induces apoptosis in a p53-independent manner in tumors of neuroectodermal origin. Experiments in Fig. 5 of their paper (1) to measure p53-induced cell death were carried out with SHEP neuroblastoma cells, investigating p53 accumulation only at 3, 6, 12, 24, and 36 h of exposure to BA. However, they had shown previously in Fig. 1 of their paper (1), a BA-mediated induction of apoptosis after a 72-h exposure to this agent. On the basis of data taken 12–36 h before apoptosis is actually induced in neuroectodermal tumors, they concluded that apoptosis mediated by 10 μg/ml of BA occurred independently of p53, because p53 was induced within 12 h in the same cells by 0.5 μg/ml Doxo. It is obvious that their cells are 20-fold more susceptible to Doxo than to BA, because they required at least 10 μg/ml of the latter to show much slower effects. However, it does not prove that p53 is not involved in BA-mediated apoptosis. As a matter of fact, a recent report from our laboratory using cells of common neuroectodermal origin, *e.g.*, matched human melanoma C8161 and Mel/Juso cell lines with differing metastatic potential, showed a BA-mediated induction of p53 in all of four cell lines and preferential apoptosis in the more metastatic melanoma types after 72 h of exposure to 2 μg/ml BA (2). We investigated recently the effect of BA on human neuroblastoma[2] and also find that apoptotic response is associated with induction of p53, with kinetics similar to that found for human melanoma cells. Our data demonstrating the relevance of p53 in BA-mediated apoptosis are in agreement with an abstract presented recently by others (3), who demonstrated BA-mediated apoptosis in Mel-2 concurrently with induction of p53 but neither apoptosis nor p53 increase in Mel 6 or Malme-3M cells.

In summary, the claim that BA triggers a p53-independent apoptosis pathway different from the one identified previously for standard chemotherapeutic drugs (1) is perhaps misleading because these conclusions may be based on: (*a*) measuring prematurely p53 induction much too early before the apoptotic response, or (*b*) using cells poorly susceptible to BA (10–50 μg/ml) because most susceptible cells respond to this agent at levels one-fifth or one-tenth lower (4).

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References

1. Fulda, S., Friesen, C., Los, M., Scaffidi, C., Mier, W., Benedict, M., Nuñez, G., Krammer, P. H., Peter, M. E. and Debatin, K-M. Betulinic acid triggers CD95 (Apo1/Fas)- and p53-independent apoptosis via activation of caspases in neuroectodermal tumors. Cancer Res., *57:* 4956–4964, 1997.
2. Rieber, M., and Strasberg Rieber, M. Induction of p53 without increase in p21WAF1 in betulinic acid-mediated cell death is preferential for human metastatic melanoma. DNA Cell Biol., *17:* 399–406, 1998.
3. Pisha, E., Pezzutto, J. M., Rauth, S., DasGupta, T. K. Melanoma cell cycle perturbations by betulinic acid. Proc. Am. Assoc. Cancer Res., *39:* 68, 1998.
4. Pisha, E., Chai, H., Lee, I. S., Chagwedera, T. E., Farnsworth, N. R., Cordell, G. A., Beecher, C. W., Fong, H. H., Kinghorn, A. D., Brown, D. M., *et al.* Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. Nat. Med., *1:* 1046–1051, 1995.

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[1] The abbreviations used are: BA, betulinic acid; Doxo, doxorubicin.
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Reply

In our recent article (1), we report that BA[1] induces apoptosis without accumulation of p53 protein in neuroectodermal tumor cells. Drs. Rieber (2) criticize that p53 induction, determined at 3, 6, 12, 24, and 36 h of exposure to BA, was prematurely measured before the apoptotic response. However, as is evident from Fig. 2, caspase activity and cleavage of caspases started 6–12 h after the addition of BA and was almost complete at 36 h (1). In addition, as shown in Figs. 4 and 5, mitochondrial perturbations occurred within the first 12 h of exposure to BA (1). Data on time dependency of BA-induced apoptosis as determined by DNA fragmentation were omitted from the report only for editorial reasons. However, the kinetics of BA-induced apoptosis were comparable with the kinetics of doxorubicin-induced apoptosis (1, 3). Thus, p53 induction was actually assessed at the time of the apoptosis response. Under the conditions studied, we did not observe p53 protein accumulation in cells that harbor wild-type p53 (SHEP neuroblastoma cells). Because the primary focus of our study was not on p53, we did not perform additional studies to definitely exclude the involvement of p53 in BA-induced apoptosis under all circumstances. However, sensitivity or resistance to BA was variably associated with loss of p53 function among the cell lines studied because p53-mutant DAOY medulloblastoma cells were responsive to BA, whereas p53-mutant HT-29 colon carcinoma cells did not respond to BA (1). Drs. Rieber report that p53 protein was increased in one of the two metastatic melanoma cell lines only when BA was administered in combination with bromodeoxyuridine but not when BA was given alone (4). In the abstract referred to by Drs. Rieber, Pisha *et al.* (5) actually found a stronger association between the efficacy of BA treatment and the p16 status compared with the p53 status.

Moreover, Drs. Rieber claim that our conclusion may be misleading because we used cells poorly susceptible to BA. However, although neuroblastoma cells may be less responsive to BA compared with melanoma cells, no marked differences were observed when comparing ED_{50}s (1–5 μg/ml for four melanoma cell lines and 3–10 μg/ml for four neuroblastoma cell lines; Refs. 1 and 6).

In summary, our conclusion that BA induces apoptosis independently of p53 in neuroectodermal tumor cells is based upon the fact that p53 protein did not accumulate at the time of the apoptosis response in sensitive neuroblastoma cells. Our data and the data quoted by Drs. Rieber do not provide clear evidence that wild-type p53 function is essentially required for BA-mediated apoptosis. How-

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[1] The abbreviation used is: BA, betulinic acid.