A Truncated Erythropoietin Receptor and Cell Death

Recently, Y. Nakamura *et al.* showed (*1*) that human bone marrow cells express both a truncated and a full-length form of the erythropoietin receptor (EPOR), with the truncated form predominating in immature progenitors and the full-length form predominating in late-stage progenitors. The title of the paper (*1*) states that the truncated form of the receptor "Fails to Prevent Programmed Cell Death . . .," but that conclusion is inconsistent with the data. When the truncated receptor is expressed in a cell line that normally undergoes programmed cell death in the absence of erythropoietin (EPO), the transfected cells' survival and growth are dependent on EPO. Compared with cells expressing the full-length receptor (EPOR-F), cells expressing the truncated receptor (EPOR-T) are more likely to undergo apoptosis at lower concentrations of EPO. Nakamura *et al.* interpret these data to mean that the short cytoplasmic tail of EPOR-T contains regions that can transduce a mitogenic signal but that it lacks regions required to efficiently inhibit programmed cell death. However, if EPOR-T fails to prevent programmed cell death, then the transfected cells should have died regardless of the EPO concentration.

I propose that EPOR-T does not selectively fail to prevent programmed cell death but, rather, that it transduces EPO actions inefficiently. This would explain why cells expressing EPOR-T do not undergo apoptosis when the EPO concentration is high. In addition, differences in transducing efficiency between EPOR-T and EPOR-F would confer differential sensitivity to EPO in early progenitors (which express predominantly EPOR-T) and in later stage progenitors (which express primarily EPOR-F). This, in turn, is consistent with the observation that the proliferation of colony-forming units in vitro requires low doses of EPO but that the proliferation of burst-forming units requires not only a source of interleukin-3 but also high doses of EPO.

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Response: Schwall raises a possibility that we cannot rule out at present. The data in figure 3B of our paper (*1*) give an impression that EPOR-T transduces the proliferation signal inefficiently at a lower concentration of EPO. However, as we described in our report, this inefficiency is at least in part a result of the poor viability of the Ba/F3(EPOR-T) cells that remain in the culture during thymidine treatment. With conventional technology, it is difficult to accurately measure proliferation of these cells, some of which are dying during culture.

The data in figure 3A of our paper (*1*) show that there was a difference in the likelihood of cell death of Ba/F3(EPOR-T) and of Ba/F3(EPOR-F) cells after they were deprived of EPO, although both transfectants had been cultured in a high concentration (10 U/ml) of EPO. Thus, even in the presence of large amounts of EPO, where both EPOR-T and EPOR-F show maximum plateau phase proliferation, EPOR-T cannot convey a signal (or signals) to prevent apoptosis of Ba/F3 cells as well as EPOR-F can. Thus, the distal cytoplasmic portion of EPOR-F seems to have an important role in the prevention of apoptosis. Others (*2*) have shown that in erythroid colony-forming unit cells, which presumably express EPOR-F dominantly, a low concentration of EPO can maintain cellular viability independent of proliferation. Functions for the distal cytoplasmic portion of EPOR (other than mitogenic signal transduction) have been suggested by several other groups that used truncated forms of EPOR from mouse EPOR deletion mutants (*3*).

Schwall states that "if EPOR-T fails to prevent programmed cell death, then the transfected cells should have died regardless of the EPO concentration." However, prevention of the apoptosis of the Ba/F3 transfectant is not totally dependent on signals from EPO and EPOR; other factors must be involved in this process. Fetal calf serum in culture medium prevents apoptotic death, and the cell cycle may regulate apoptosis. It appears that, when the cells are in maximum growth, they do not die of apoptosis, but when the cells are not proliferating maximally, they may die without the signal to prevent apoptosis. Thus, the signal mediated by the cytoplasmic portion of EPOR determines the proneness to cell death under a low (physiological) concentration of EPO. Once the EPO concentration becomes high (for example, from sudden bleeding), the immature erythroblast that expresses EPOR-T can proliferate quickly by escaping from apoptotic death.

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