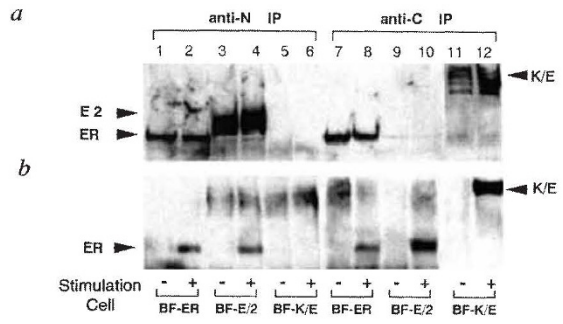
I found that BF-ER and BF-E2 cells grew in Epo whereas BF-K/E cells grew in stem cell factor (SCF) as reported [2,4]. As illustrated in the figure, an antiserum against the extracellular domain of the Epo receptor (anti-N) precipitated the M_r 66,000 (66K) Epo receptor (panel *a*, lanes 1,2) and the 70K–75K E2 (lanes 3, 4) but not kit/ER (lanes 5,6) whereas an antiserum against the cytoplasmic domain (anti-C) precipitated the Epo receptor (lanes 7, 8) and kit/ER (lanes 11, 12) but not E2 (lanes 9,10). With both antisera,



BF-ER and BF-E2 cells were stimulated with Epo and BF-K/E cells with SCF. Anti-N and anti-C immunoprecipitates (IP) from unstimulated (–) and stimulated (+) cells were immunoblotted with anti-N (*a*, lanes 1–10), anti-C (*a*, lanes 11 & 12) or anti-phosphotyrosine (PY) (*b*). Arrowheads, position of the wild-type Epo receptor (ER), the E2 chimaera (E2) and the kit/ER chimaera (K/E).

however, I detected a protein in BF-E2 cells that was the size of the wild-type receptor, consistent with the expression of the endogenous Epo receptor in these cells.

The expression of a wild-type Epo receptor suggests that the results of Chiba *et al.* can be explained by signalling through the wild-type receptor rather than the chimaeric E2 receptor. To explore this possibility, I next examined Epo-induced tyrosine phosphorylation of the chimaeric receptors as a marker for their use. Epo stimulation of BF-ER cells resulted in tyrosine phosphorylation of the Epo receptor (panel *b*, lanes 2, 8) as expected, while SCF stimulation of BF-K/E cells resulted in tyrosine phosphorylation of the kit/ER chimaera (lane 12). With BF-E2 cells, Epo stimulation did not induce detectable tyrosine phosphorylation of the E2 chimaera but did induce the tyrosine phosphorylation of the endogenous, wild-type receptor as detected by both anti-N and anti-C (lanes 4, 10). This indicates that the endogenous Epo receptor in BF-E2 cells is functionally expressed on the cell surface. This result also raised a concern about the functional integrity of the E2 chimaera. The level of tyrosine-phosphorylation of the wild-type receptor in BF-E2 cells was comparable to that in EF-ER cells, suggesting that the amount of cell-surface wild-type Epo receptor in BF-E2 cells was similar to that in BF-ER cells. This might be due to an erythroid-like phenotype of BF-E2 cells[5], because the endogenous Epo receptor in erythroid cells was efficiently expressed on the cell surface[6], whereas ectopically overexpressed Epo receptor was mostly present inside the cells.

The results demonstrate that BF-E2 cells express and use the endogenous Epo receptor, and can explain the results by Chiba *et al.*, without hypothesizing another signal transducer. The basis for the high levels of expression of the endogenous receptor in BF-E2 cells is unknown. Because the parental cells barely express Epo receptor, it is likely that expression of the endogenous receptor is activated during selection. Alternatively, expression of the E2 chimaera may affect the expression or stability of the endogenous Epo receptor.

The above results may also bring into question the results by Chiba *et al.* with a chimaeric receptor (2E-R) containing the extracellular domain of the IL-2 receptor β-chain and the cytoplasmic domain of the Epo receptor. In particular, studies by Mori *et al.*[7] had found that this chimaera, while binding IL-2, could not transmit a proliferative signal while Chiba *et al.* found that it could. It is possible that the endogenous IL-2 receptor β-chain was activated in the cells examined by Chiba *et al.* This is a particularly important point in view of the recent demonstration that the IL-2 and Epo receptors associate with and activate different Janus kinases[8].

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TODOKORO REPLIES — Erythropoietin (Epo)-induced receptor phosphorylation cannot be detected by an immunoblotting of the total cell lysates with anti-phosphotyrosine antibody, because the level of receptor phosphorylation is too low, and thus the chimaera receptor phosphorylation was not shown in our paper[4]. Yoshimura claims that Ba/F3 cells expressing the E2 chimaera (BAF/E2) induce the expression of endogenous Epo receptor and thus that Epo stimulation induces phosphorylation of the Epo receptor, although it is not clear why endogenous receptor expression is induced.

In our hands, however, we cannot reproduce his results. As we have previously described, the E2 chimaera[5] as well as the Epo receptor[5,9] in Ba/F3 cells transmit an erythroid-specific differentiation signal, that is, they induce the expression of the erythroid-specific transcriptional factors GATA-1 and SCL, which in turn induce expression of erythroid-specific genes such as globin and the Epo receptor. Therefore, once BaF/E2 cells as well as BaF/Epo receptor cells are stimulated with Epo, they are committed to erythroid-like cells expressing erythroid-specific proteins including the Epo receptor, and this process is irreversible. Yoshimura observed the endogenous Epo receptor because he used BaF/E2 cells cultured with Epo. But when we added Epo to transfectants that had not been cultured with Epo, we observed that both growth and differentiation signals were transmitted through the chimaera receptors but not through wild-type Epo receptor.

We also showed that the parental cells or the transfectants expressing inactive mutant Epo receptors would never spontaneously grow in response to Epo through the endogenous Epo receptor. Our previous papers simply suggested a possibility that there exists another subunit for Epo receptor[4,5]. I agree with Yoshimura that homodimerization of the Epo receptor is necessary for signal transduction, but there is no evidence that it alone is sufficient. No one can explain why the crosslinking of the labelled Epo with Epo receptors always shows multiple bands, only one of which reacts to anti-Epo receptor antibody[10]; therefore, the possibility of a second subunit cannot be ruled out. There are several pieces of evidence supporting the existence of membrane proteins associated with Epo receptor[10–17], but in order to clarify this issue the second subunits must be isolated and characterized.

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