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How Signaling Promotes Stem Cell Survival: Trophoblast Stem Cells and Shp2

Trophoblast stem (TS) cells require FGF4 for self-renewal and to prevent differentiation. In this issue of *Developmental Cell,* Yang and colleagues show that the tyrosine phosphatase Shp2 prevents apoptosis in TS cells, by activation of Erk and subsequent phosphorylation and destabilization of the pro-apoptotic protein Bim. These studies provide a novel link between FGF/Erk signaling and cell survival that may be relevant to other stem and progenitor cell niches.

Trophoblast stem (TS) cells can be derived from the placental lineage of the developing mouse during the early stages of development (Tanaka et al., 1998). Like other stem cells, TS cells have the ability to self-renew or to differentiate into more specialized, lineage-specific cell types, depending on reception of appropriate signals. Among these, fibroblast growth factor-4 (FGF4) is required to repress differentiation and ensure stem cell self-renewal. In this issue of *Developmental Cell*, Yang and colleagues provide a link between FGF signaling and survival of TS cells and their in vivo progenitor, the trophoblast. By examining the requirement for the tyrosine phosphatase Shp2 both in vivo and in TS cells, the authors present a model in which Shp2 promotes embryonic survival at least in part by promoting FGF4-dependent Erk signaling and the suppression of Bim-dependent apoptosis in the trophoblast (Yang et al., 2006).

FGF signals are mediated by FGF receptors (FGFR1–4), which are receptor tyrosine kinases (RTKs). Ligand stimulation of RTKs leads to phosphorylation of tyrosine residues on the intracellular region of the receptor that recruits downstream signaling proteins via specialized protein binding domains. Among the downstream signaling molecules are cytosolic tyrosine kinases and phosphatases that regulate cellular responses to ligand stimulation. One class of tyrosine phosphatase is the so-called SH2 phosphatases, Shp1 and Shp2, which are orthologous to *Drosophila* Corkscrew and *Caenorhabditis* Ptp2. While Shp1 appears to act as a dedicated antagonist of RTK signaling, mounting evidence suggests that Shp2 promotes RTK signaling.

Like other RTKs, FGF signaling can activate the Erk pathway. Mutations in members of the FGF and Erk pathways have demonstrated their importance in development of extraembryonic tissues such as the trophoblast. Mutations in *Fgf4*, *Fgfr2*, *Frs2*α, and *Erk2* all lead to lethality soon after implantation, consistent with trophoblast proliferation defects (Arman et al., 1998; Feldman et al., 1995; Gotoh et al., 2005; Saba-El-Leil et al., 2003). These and other data have lent support to the hypothesis that FGF4 produced by the embryo provides a niche for TS cells in the neighboring trophoblast (Figure 1). Trophoblast cells within range of FGF4 continue to proliferate and retain the ability to form TS cell colonies in vitro, while trophoblast cells outside of the range of this signal differentiate into giant cells and other trophoblast derivatives.

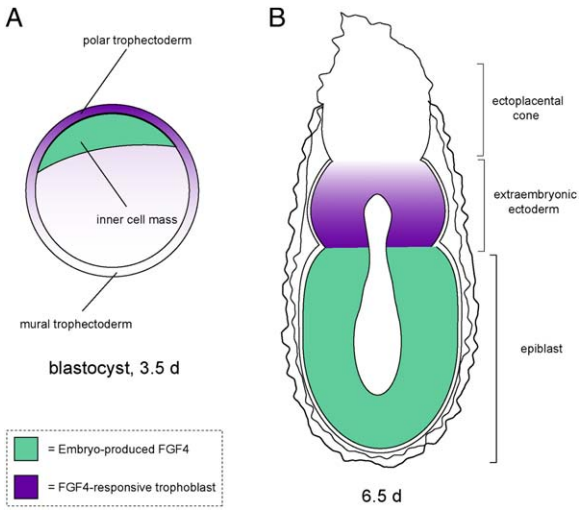

Figure 1. FGF4 and the TS Cell Niche

(A) FGF4 is initially expressed in the inner cell mass, the embryonic lineage. Cells of the polar trophectoderm are within range to receive FGF4, whereas the mural trophectoderm is out of range.

(B) At 6.5 days of development, FGF4 produced in the epiblast is received by cells of the neighboring extraembryonic ectoderm, while cells of the ectoplacental cone are out of range. TS cells can be derived from 3.5 and 6.5 day trophoblasts.

In this study, Yang and colleagues add a new player and a new twist to FGF signaling in the trophoblast. They present evidence that Shp2 is required for embryo survival far earlier than previously reported based on analysis of other targeted alleles and that this requirement is at least partially due to the role of Shp2 in ensuring trophoblast survival. A proven null mutation of *Shp2* leads to lethality around the time of implantation. Blastocyst development occurs but blastocyst outgrowths show little differentiation and much apoptosis. Levels of phospho-Erk are also reduced in mutant blastocysts. Perhaps not surprisingly, TS cells cannot be derived from *Shp2* mutants. To get around this, the authors make clever use of TS cells carrying floxed alleles of *Shp2.* Infection of these cells with adenovirus expressing the Cre recombinase leads to efficient deletion of *Shp2*, allowing phenotypic analysis of the effects of loss of *Shp2* in TS cells. Loss of *Shp2* leads to the failure of TS cells to proliferate and induces apoptosis.

TS cells provide an ideal system for examining signaling in trophoblast proliferation, and they have recently extended our understanding of FGF signaling in trophoblast. FGF4 causes phosphorylation of FGFR2 in TS cells (Gotoh et al., 2005; Yang et al., 2006), formation of a Grb2/FRS2α/Shp2 complex, and phosphorylation of Erk (Gotoh et al., 2005). *Shp2* knockout TS cells contain lower levels of activated Ras and Erk, consistent with the results of in vivo loss of Shp2. Interestingly, Src family kinases (SFKs), which require dephosphorylation of

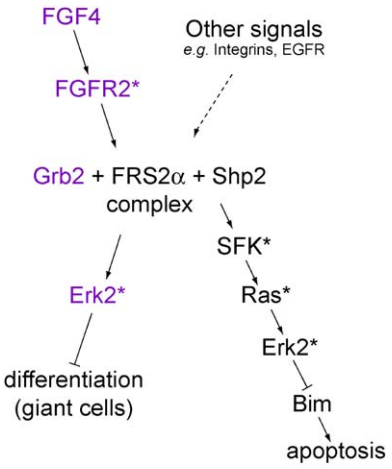

Figure 2. Signaling Prevents Differentiation and Apoptosis in TS Cells

Shp2 is required for activation (asterisk) of SFKs, Ras, and Erk2, which inhibits Bim-dependent apoptosis (Yang et al., 2006). Other members of this pathway (shown in purple) play a role in preventing differentiation, since giant cells form in their absence (Feldman et al., 1995; Arman et al., 1998; Cheng et al., 1998; Saba-El-Leil et al., 2003). Shp2 may transduce other signals and serve as a branchpoint between antiapoptotic and antidifferentiation programs that, together, ensure continued self-renewal of TS cells.

specific tyrosine residues for activation, are poorly dephosphorylated in *Shp2* knockout cells. SFK inhibitors also inhibit activation of Ras and Erk in TS cells. Together these data suggest that SFK dephosphorylation by Shp2 mediates Ras/Erk signaling in TS cells.

The proapoptotic protein Bim is known to be regulated by Erk phosphorylation, which leads to its destabilization and degradation. Bim is rapidly degraded in wild-type TS cells stimulated with FGF4, but is not degraded in $Shp2^{-/-}$ TS cells, offering a molecular explanation for the apoptotic phenotype. Introduction of a short-hairpin RNA targeted against *Bim* into $Shp2^{-/-}$ TS cells reduced apoptosis and allowed some recovery of TS cell proliferation. This suggests that Shp2 suppresses apoptosis by activating the Erk pathway to phosphorylate Bim and that this is an important part of the mechanism driving normal TS cell self-renewal. What is not clear is whether this is the only way that FGF signaling through the Erk pathway can affect TS cell maintenance. As the authors themselves point out, withdrawal of FGF4, which causes failure of TS cell proliferation, does so by promoting TS cell differentiation, not by inducing cell death (Tanaka et al., 1998). Mutation of other FGF/Erk pathway members interferes with derivation of stable TS cell lines, but does not prevent differentiation of trophoblast cells in vitro (Figure 2). This suggests that there must be other, Shp2-independent but Erk-dependent pathways downstream of FGF that regulate proliferation versus differentiation in TS cells. Shp2 may represent a branchpoint between antiapoptosis and antidifferentiation programs in TS cells (Figure 2). It is also possible that some of the effects of *Shp2* mutation in TS cells are in signaling pathways other than FGF, since TS cells cannot be maintained by FGF alone.

Intriguingly, FGF is thought to play its antiapoptotic role via activation of Akt, not via the Erk pathway (Mao and Lee, 2005). The novel involvement of Shp2 and Erk in preventing apoptosis in proliferating stem cells may be relevant to other in vivo situations where FGF plays a role in maintaining proliferating stem and progenitor cells. For example, FGF-dependent neural stem cells in the brain show increases in cell number when treated with inhibitors of cell death (Morshead and van der Kooy, 2000). In the limb bud and branchial arches, FGF signaling is required for proliferation and outgrowth of the mesenchymal progenitors. *Shp2* mutant cells cannot contribute to these proliferating regions in chimeras (Saxton et al., 2000), a result that was interpreted as a failure of cell proliferation but could well involve induction of apoptosis. Understanding how FGF signaling impacts proliferation, differentiation, and apoptosis in a simple system like TS cells should help us understand signaling interactions in many different stem and progenitor cell niches.

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