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PERSPECTIVE

Live Long and Prosper

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ABSTRACT

In this issue of *Molecular Pharmacology*, Meyn et al. (p. 1320) provide the results of the first comprehensive investigation of the expression pattern of the Src family of nonreceptor tyrosine kinases (SFK) in mouse embryonic stem (ES) cells. They found that self-renewing ES cells express seven of the eight mammalian SFK members and that some undergo distinct expression changes during early differentiation events. One of the most dramatic changes was in Hck transcript levels, which decreased almost 30-fold during the first 3 days of embryoid body formation, a culture system model of early embryogenesis and differentiation. Other SFKs, such as Fyn and Src, remain present and active as ES cells differentiate. Of particular interest was the observation that ES cell self-renewal or differentiation can be manipulated through the selective pharmacological inhibition of SFK members. This information should help in the expanding efforts to exploit ES cells for basic and clinical purposes.

We all begin modestly when our father's sperm meets our mother's egg. To live long and prosper, we must employ unspecialized precursor cells or stem cells, which have the unique ability to self-renew and differentiate into specialized cells in response to appropriate signals. These stem cells are categorized as either embryonic or tissue-specific; embryonic stem (ES) cells are derived from the inner cell mass of the early embryo (blastocyst) and tissue-specific stem cells (adult stem cells) are unspecialized cells found in differentiated tissues. ES cells can give rise to any differentiated cell type derived from the three primary germ layers of the embryo (endoderm, mesoderm, and ectoderm), as well as germ cells. Because of this remarkable developmental potential, ES cells hold substantial therapeutic promise for transplantation and regenerative medicine (Keller, 2005).

ES cells generally can be maintained in culture in an undifferentiated state indefinitely without losing differentiation potential. When injected into a host, they will produce a benign teratoma containing multiple tissue types. Therefore, it is likely that ES cells will need to be differentiated into the desired tissue and subtype-specific cells before they can be used clinically. Seeking methods to maintain "stemness", while permitting expansion of the cells and guiding their differentiation along a desired lineage before transplant, has been a major challenge. Most investigators have used 'cocktails' of growth factors, signaling molecules, or genetic manipulation (Keller, 2005; Wobus and Boheler, 2005), but others have recently begun to use small organic molecules to influence stem cell behavior (Ding and Schultz, 2004). As we start to understand the central signaling pathways controlling ES cell self-renewal and differentiation, it should be possible to identify rational molecular targets for small molecule intervention. In the current issue, Meyn at al. (2005) have performed the first systematic analysis of the Src family of protein-tyrosine kinases (SFKs) in ES cells undergoing differentiation and have used SFK inhibitors to decode the participants in this process.

Early evidence implicating SFKs in ES cell signaling comes from the work of Ernst et al. (1994, 1996, 1999). These studies implicated the SFK member Hck in signal transduction by leukemia inhibitory factor (LIF), which is used to maintain ES cell pluripotency in feeder-free cultures (Smith et al., 1988; Williams et al., 1988). The SFK c-Yes has also been implicated in the maintenance of ES

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ABBREVIATIONS: ES, embryonic stem; EB, embryoid bodies; SFK, Src family of protein-tyrosine kinase; LIF, leukemia inhibitory factor; SU6656, 2-oxo-3-(4,5,6,7-tetrahydro-1*H*-indol-2-ylmethylene)-2,3-dihydro-1*H*-indole-5-sulfonic acid dimethylamide; PP2, 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine.

cell pluripotency; suppression of Yes protein expression results in ES cell differentiation in the presence of LIF (Anneren et al., 2004). There is, however, little information about the expression of other SFKs in ES cells and how the expression of each family member is regulated during the earliest stages of differentiation. Meyn et al. (2005) found that seven of the eight mammalian SFKs are expressed in self-renewing ES cells. Several of these SFK members undergo dynamic changes in transcriptional regulation during differentiation to embryoid bodies (EBs), an in vitro culture system that models early embryogenesis (Keller, 1995). Most dramatic were changes in Hck transcript levels, which decreased almost 30-fold during the first 3 days of EB formation. Lck message levels also decreased, but they did so more slowly and at a rate that mimicked the loss of ES cell pluripotency. Although the remaining family members showed relatively constant transcript levels in renewing and differentiated ES cells, only Src and Fyn showed constitutive kinase activity.

Based on these results, it seems reasonable to hypothesize that a subset of SFKs independently control pathways linked to self-renewal and differentiation. Studies with pharmacological inhibitors that show different selectivity profiles for the individual SFK members support this view. It is surprising that complete nonselective inhibition of SFK activity with either PP2 or A-419259, ATP-competitive inhibitors, slowed growth and prevented differentiation. This block in differentiation is reversible and is only observed if the cells are treated 3 to 4 days after the start of differentiation. Some might conclude that these results contradict previous published work (Anneren et al., 2004), which demonstrated that the SFK inhibitor SU6656 caused ES cells to undergo differentiation in the presence of LIF. Meyn and colleagues show, however, that SU6656 treatment resulted in only a partial inhibition of SFK activity in ES cells and hypothesize that inhibition of a subset of SFKs linked to renewal (e.g., Hck or Yes) may result in differentiation, whereas complete inhibition of SFK activity interferes with differentiation. Other work has shown that SU6656 is particularly active against Yes relative to other SFKs in vitro (Blake et al., 2000).

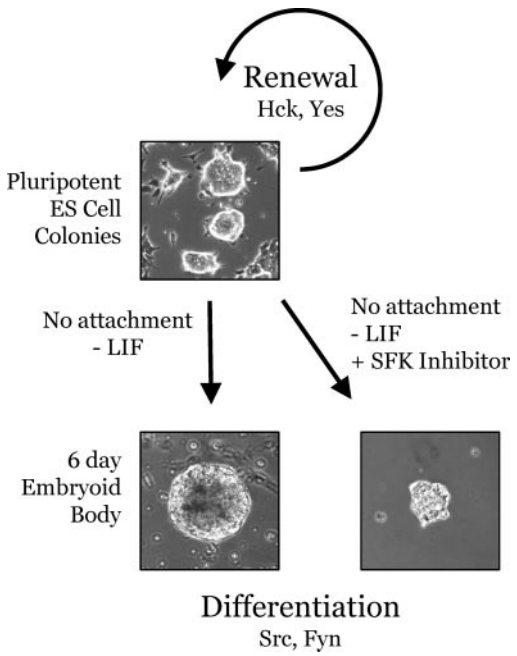

Fig. 1. Regulation of ES cell renewal and differentiation by SFKs. Murine ES cells grow as tight colonies in the presence of LIF, which is required to suppress differentiation and maintain pluripotency (top). The SFKs Hck and Yes have been implicated in the maintenance of self-renewal. Plating cells under conditions in which they are unable to attach and in the absence of LIF promotes the formation of embryoid bodies (EBs), structures that model the early stages of embryogenesis. Both c-Src and Fyn continue to be expressed and remain active during EB formation. A 6-day EB is shown on the lower left. When EBs are formed in the presence of inhibitors that block all SFK activity, growth and differentiation are arrested, resulting in smaller EBs that retain pluripotent cells (lower right; EBs are shown at the same magnification).

The 4-anilinoquinazoline SFK inhibitor SKI-1 provides pharmacological support for an emerging model in which self-renewal and differentiation are controlled by different SFKs (Fig. 1). Using classic concentration-response approaches, the authors achieve a range of SFK inhibition, from partial to complete. Partial inhibition of SFK activity causes ES cell differentiation, mimicking the results with SU6656, whereas complete inhibition of SFK activity with higher SKI-1 concentrations blocks differentiation, mimicking the effects of A-419259 and PP2. It is interesting that analysis of SKI-1 IC_{50} values against purified SFKs in vitro reveal this inhibitor to be more active against the putative "self-renewal" kinases Hck and Yes than against Fyn and Src, which remain active as ES cells differentiate to embryoid bodies. The implication is that the activity of Src and Fyn are essential for embryoid body formation.

In summary, the article by Meyn et al. (2005) supports the ideas that individual members of the Src kinase family control discrete aspects of stem cell behavior and that individual SFKs represent targets for the manipulation of stem cell fate with small molecule inhibitors in culture. The conserved structural architecture of SFK domains may make the design of isoform-selective inhibitors difficult (Boggon and Eck, 2004), but we know from experience with inhibitors of other protein tyrosine kinases, such as the Brc-Abl inhibitor imatinib, that specificity can be achieved (Capdeville et al., 2002). Compounds with even a modest selectivity profile, as illustrated with SKI-1, might have a major impact on the cellular response and could be useful for basic pharmacological studies possibly in the future for regenerative and transplantation medicine.

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