FINAL NARRATIVE REPORT

1. Original aims of the project:

The overall goal of the project is to elucidate the mechanisms by which EPO affects neural stem cells for therapeutic purposes. To achieve this goal we originally planned to pursue the following three specific aims: 1. to examine EPO effects on survival, proliferation and differentiation of neural stem cells in vitro; 2. to decipher the mechanisms of EPO effects on neural stem cells; 3. to investigate EPO effects on transplanted neural stem cell survival and differentiation in injured spinal cords.

2. Project successes:

Specific aim 1: to examine EPO effects on survival, proliferation and differentiation of neural stem cells in vitro

We found that EPO promotes survival or proliferation of neural stem cells, however, at relatively high concentration, which may not be physiologically relevant. On the other hand, we found that another drug, lithium, promotes proliferation but not survival of neural stem cells. Lithium also stimulates neuronal differentiation of neural stem cells upon differentiation induction.

Specific aim 2: to decipher the mechanisms of EPO effects on neural stem cells

We found that in neural stem cell-like cells, EPO activates a potent cell survival/proliferation pathway, the ERK pathway, which may through a c-Raf independent mechanism. In addition, we found that EPO selectively up-regulates RNA expression of anti-apoptotic gene Bcl-w but not Bcl2 or Bcl-xL in the neural stem cell-like cells.

We also investigated the mechanism by which lithium stimulates neural stem cell proliferation. Our results suggest that lithium's stimulation of neural stem cell proliferation does not involve neurotrophic factor stimulation, or inositol depletion, but may involve GSK-3β inhibition and subsequent NFAT activation.

Specific aim 3: to investigate EPO effects on transplanted neural stem cell survival and differentiation in injured spinal cords

We found that in injured rat spinal cords, EPO stimulates RNA expression of EPO receptor and nestin, two proteins that are expressed in neural stem cells.

These results suggest that EPO may stimulate endogenous neural stem cells in the spinal cord to benefit spinal cord injury. Amazingly, we also found that lithium robustly promotes survival and possibly proliferation of neural stem cell-like cells transplanted into injured rat spinal cords. In addition, lithium treatment remarkably stimulates RNA expression of several neurotrophic factors in the injured rat spinal cords transplanted with these neural stem cell-like cells.

3. Project challenges:

Our studies suggest that lithium stimulates neural stem cell proliferation through GSK-3β inhibition. GSK-3β modulates activity of a plethora of enzymes. It is hard to know which enzyme is involved in lithium's promotion of neural stem cell proliferation. Using gene array analysis, we found that NFAT activation is most likely involved. We found that CsA, a specific inhibitor of the major NFAT activator calcineurin, completely abolished lithium's effect on neural stem cell proliferation, suggesting that NFAT activation contributes to lithium's promotion of neural stem cell proliferation.

4. Significance:

Our studies suggest that EPO may promote neural stem cell survival and possibly proliferation through ERK pathway activation and Bcl-w up-regulation, and may stimulate endogenous neural stem cells in the spinal cord after injury. In addition, our studies revealed that lithium stimulates neural stem cell proliferation but not survival through GSK-3β inhibition and subsequent NFAT activation, and that lithium promotes neuronal differentiation of neural stem cells. Furthermore, our studies showed that lithium robustly promotes survival of neural stem cell-like cells transplanted into injured rat spinal cords, and stimulates several neurotrophic factor RNA expression in injured rat spinal cords transplanted with these cells. These studies not only provide important insight into the physiological functions of erythropoietin and the beneficial effects of erythropoietin and lithium in the central nervous system (CNS), but also suggest new ways of improving survival or proliferation of cells transplanted into the CNS, and stimulating neurotrophic factors in the CNS to benefit CNS injury.

5. Plans:

Our results suggest that erythropoietin does not promote survival or proliferation of neural stem cells at doses that improve neurological functions in spinal cord injury, but does so at higher doses. This suggests that other cells in the CNS may facilitate the EPO effects on neural stem cells. We could

determine which type(s) of cells facilitates EPO effects on neural stem cells by co-culturing neural stem cells with different cell types in the CNS.

Our results showed that lithium robustly promotes survival or proliferation of neural stem cell like-cells transplanted into injured rat spinal cords, and remarkably stimulates neurotrophic factor RNA expression in the injured rat spinal cord with transplants of these neural stem cell-like cells. Next we would like to know what potential beneficial effects the combined treatment of lithium with neural stem cell transplantation could have on spinal cord injury, for example, lesion volume reduction, reduced cell death, reduced cavity formation, reduced glial scar formation, functional recovery improvement, etc. We also would like to explore whether lithium promotes survival or proliferation of transplants of other types of cells, and whether lithium combined with transplantation of these cells could benefit spinal cord injury.

6. Publications:
   1) **Qu Z**, Sun D, Adamson C, Tanna M, Young W. Erythropoietin promotes neural stem cell survival or proliferation: the involvement of ERK pathway activation and Bcl-w up-regulation. (In preparation)
   2) **Qu Z**, Sun D, Young W. Erythropoietin stimulates expression of its receptor and neural stem cell marker nestin in the injured spinal cords. (In preparation)
   3) **Qu Z**, Sun D, Young W. Lithium promotes neural precursor cell proliferation: evidence for the involvement of the non-canonical GSK-3b-NF-AT signaling. (In preparation)
   4) Iseda T, Hashimoto M, **Qu Z**, Sun D, Young W. Lithium robustly increases numbers of neural stem cell like-cells transplanted into injured rat spinal cord. (In preparation)
   5) Iseda T, Hashimoto M, **Qu Z**, Sun D, Young W. Lithium treatment combined with transplantation of neural stem cell-like cells stimulates neurotrophic factor expression in injured rat spinal cords. (In preparation)