Deciphering cell-cell communication in the developing mammalian brain

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The diverse subtypes of neurons that comprise the mammalian cerebral cortex are produced from a single population of cortical neural precursor cells during the period of embryonic neurogenesis. While this process of neurogenesis is tightly controlled at the transcriptional and translational levels, substantial opportunity exists for extrinsic or niche control of the process of neurogenesis. In our recently published work we made use of a combination of computational and biologic approaches to characterize cell-cell communication between cortical neurons and cortical precursor cells and thereby reveal an unexpectedly complex growth factor communication network that accurately predicted new regulators of cortical neurogenesis.

The cerebral cortex comprises diverse subtypes of projection neurons with cell bodies that are organized in a stereotypic laminar fashion, and axons that project both within the cortex itself and to more distant areas of the brain. The production of this high level of neuronal diversity is accomplished during the embryonic neurogenic period, which begins during mid-gestation (roughly gestational day 11 in the mouse) and continues until near birth when, through a process we are just now starting to understand, the correct numbers and types of neurons are generated. Remarkably, all of these different types of cortical neurons are produced by a population of cortical radial precursor cells (also called radial glial cells) that line the lateral ventricles. These radial precursors either divide symmetrically to self-renew and expand their numbers, or asymmetrically to produce neurons directly or via a neurogenic transit-amplifying cell called an intermediate progenitor. Work performed by many laboratories, including our own, has established that this process of embryonic cortical neurogenesis is tightly controlled at both the transcriptional (reviewed in ref. 2) and translational levels. However, far less attention has been focused on how secreted growth factors and cytokines regulate cortical precursor biology, even though it has long been known that appropriate cortical neurogenesis is dependent upon the developing neural environment. This relative paucity of information is likely due to limitations vis a vis experimental approaches, and because the developing cortex, like the rest of the developing brain, is a dynamic, rapidly-evolving tissue. Nonetheless, it is critical to define and understand these extrinsic cues, since they play a key role in ensuring that the right numbers and types of cells are generated, and since deficits in cell genesis are known to ultimately result in functional perturbations in neural circuitry.

During early stages of murine corticogenesis, the cortex is almost exclusively comprised of proliferating neuroepithelial precursor cells that self-renewal to increase in number. These neuroepithelial stem cells then transition to radial precursor cells that maintain their apical epithelial nature at the interface with the cerebrospinal fluid in the lateral ventricles, and extend processes to maintain their basal interface with the pial surface. These radial precursor cells start to generate neurons that migrate basally along the radial processes to form the cortical plate, which is largely comprised of newborn neurons. During late embryogenesis, radial precursors stop making neurons and start to make glia, which they will continue to make during postnatal life. A further subpopulation of these cortical radial precursors will persist to populate, in part, the adult subventricular zone neural stem cell.
niche. What then is the environment of the embryonic cortex during the period of neurogenesis? During early neurogenesis, the cortex is largely comprised of radial precursor cells and newborn cortical neurons (Fig. 1), although there are also other potential sources of secreted ligands, including the cerebrospinal fluid, the rapidly forming vasculature, and cells that start to migrate in, such as newborn interneurons from the ganglionic eminence. However, the predominant cells are the radial precursors and newborn neurons, and we therefore hypothesized that during this developmental window, the cortical growth factor environment is largely determined by these two cell types. In our very recently published work, we provided support for this hypothesis, and in the course of our studies, obtained a global overview of the growth factor environment during cortical neurogenesis. Here, we will discuss those studies and their implications for our understanding of neural precursor cells and neural development.

One of the first questions we asked in this work was whether cortical radial precursors and/or neurons actually secreted factors that could influence neurogenesis. To do this, we performed a series of culture studies that led to the conclusion that cortical precursors secreted factors that promote their own self-renewal, while newborn cortical neurons secrete factors that direct cortical precursors to produce more neurons (Fig. 1). So what were these secreted proteins? To address this question on a global level we deployed a novel computational technique that makes use of transcriptomic data to identify ligand and receptor expression and to thereby make predictions about potential cell-cell communication events. We also

Figure 1. Schematic of cell-cell communication events in the embryonic cerebral cortex during early neurogenesis. During the early neurogenic period in the embryonic cortex, the predominant cell types are the radial precursor cells (RPs), which populate the Ventricular/Subventricular Zones adjacent to the lateral ventricles, and newborn cortical neurons (Neurons) that migrate along the radial precursor basal processes to populate the Cortical Plate. In our recent paper we showed that radial precursors produce secreted factors that act in an autocrine fashion to promote their own self-renewal, while newborn cortical neurons produce secreted factors that act in a paracrine fashion to enhance the genesis of neurons from radial precursors. We modeled these potential cell-cell communication networks, and identified 3 growth factors, GDNF, NRTN and IFNγ, that promote neurogenesis. At later stages, when neurogenesis is complete, cortical neurons secrete factors like Cardiotrophin-1 that then act to promote astrogenesis.
used mass spectrometry to define the cell surface proteome on these two cell types, and thereby refine our communications model. The resultant combined model was surprisingly complex, predicting multiple autocrine and paracrine growth factor interactions, many of which had not previously been considered within the context of mammalian neural precursors. However, one caveat of this model was that all of the analyses used to build it came from cultured precursors and neurons. Thus, a key question was whether or not this complex communication model was really valid for the cortex in vivo. To address this issue, we took two approaches. First, we validated the expression of these ligands and their receptors within the embryonic cortex. Importantly, virtually all of the ligands expressed in the embryonic cortex during the neurogenic period were predicted in our precursor-neuron communication model, validating our assumption that the growth factor environment of the embryonic cortex was largely generated by these two cell types. Second, we performed a series of functional cell biologic experiments in culture and in vivo, focusing on a subset of predicted ligands that had not previously been studied within the context of cortical neurogenesis. These latter analyses further validated our communications model and identified three “new” proneurogenic ligands, glial-cell derived neurotrophic factor (GDNF), neurturin (NRTN) and interferon-γ (IFNγ).

Thus, our work developed and validated an unbiased approach for obtaining a global overview of cell-cell communication networks within the embryonic cortex, an approach that presumably will be equally valid for any developing tissue. In addition, our work not only identified three new proneurogenic ligands, but it defined a complex growth factor environment with many ligand-receptor interactions that have not been previously considered within this context. Finally, from a broad perspective, these findings support the idea that the extrinsic control of mammalian neural precursor cell biology is much more complex than previously envisioned by work focusing on single secreted growth factors. How then do we make sense of this complexity? One clue comes from our finding that when we combined multiple growth factors, neurogenesis was robustly enhanced relative to any single ligand by itself. This result suggests two, not mutually exclusive, models. First, there may be multiple pathways operative in individual precursor cells that together control neurogenesis, and it is only when these pathways are coincidentally activated by multiple ligands that the cell will generate a neuron. A mechanism like this would allow a precursor cell to sum the totality of its environment before committing to differentiation. Second, there may be subpopulations of radial precursor cells that each respond to different proneurogenic growth factors as a consequence of different receptor repertoires. In this model, each individual ligand would recruit a different subpopulation of precursors, and thus multiple ligands would result in more neurons being generated than any single ligand.

Regardless of which of these models most accurately reflects the situation in the embryonic cortex, they provide important frameworks for thinking about the multitude of intriguing questions that arise as a consequence of the predicted complexity of the cortical growth factor environment. For example, how do developing neural precursor cells interpret the “sea of growth factors” that they are exposed to and decide to self-renew and/or generate a particular type of neuron? Are neural precursor cells heterogeneous with regard to their growth factor receptor repertoires and does this have functional consequences for the types of progeny they generate? What are the receptor-activated downstream pathways that promote self-renewal versus differentiation, and how is the relative importance of these pathways determined within a single cell? Is the growth factor environment of adult neural precursor cells equally complex, as suggested by a recent paper? Answering these questions will require much future work but these answers have the potential to transform how we think about the extrinsic control of embryonic neural precursor cells, and about cell fate selection in general. In this regard, we suggest that it is the combination of functional biologic analyses together with the types of iterative computational and systems biology approaches that we deployed in our recent work that will allow us to answer these questions and that will provide an unbiased, global platform for unraveling the cell-cell communication networks that underlie the genesis and function of neural circuitry.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.
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