

## **Sunday Mornning (8am-Noon)**

### **Primary Cilia**

*Continental Room of the Sun Valley Inn*

#### **(1) The Primary Cilium: What once did nothing, now does everything.**

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Primary cilia are microtubule-based organelles that exist in almost every mammalian cell type and function as sensors of extracellular signals. In cycling cells, they grow from the mother centriole of the centrosome during early G12 and are lost before mitosis. However, the mechanisms that govern primary cilium formation are largely unknown.

Primary cilia play important roles in developmental processes such as kidney tubule patterning and the establishment of left-right axis asymmetry and have been implicated in sensing signalling molecules including Shh and PDGF. The primary cilium grows from the centrosome, which is the microtubule organizing centre in most mammalian cell types and contains two centrioles, a mother centriole and a daughter centriole, surrounded by pericentriolar material. These centrioles each nucleate the growth of a new daughter centriole during S phase such that during G2, each cell has two centrosomes, one containing the original mother centriole and one containing a new mother centriole. Between S phase and mitosis, the new mother centriole undergoes a maturation process during which it gains a set of mother centriole-specific proteins and appendages. Because centrosomes are segregated at the spindle poles during mitosis, every cell division is asymmetric with respect to mother centriole age: one sister cell receives the centrosome with the older mother centriole, while the other receives the centrosome with the new mother centriole.

Asymmetry in the fate of sister cells after division is essential for tissue differentiation during development and is important for the maintenance of stem cell identity. Asymmetric protein localization is a hallmark of asymmetric cell division, but there is evidence that organelles and DNA also segregate non-randomly in some cell divisions. Interestingly, during asymmetric stem cell division in the *Drosophila* male germ line, the older centrosome remains in the stem cell, while the younger centrosome is segregated to the differentiating cell. However, it is unknown whether the segregation of differently aged centrosomes determines phenotypic differences between sister cells.

We report that in sister cell pairs, one cell grows a primary cilium before the other, and that this asymmetry is independent of cytoplasmic differences between sister cells. We also show that the older mother centriole usually generates a primary cilium first, and that the timing of centriole maturation relative to cell cycle progression is an important factor in this asymmetry. Finally, we show that two ciliary proteins required for development, inversin and PDGFR $\alpha$ , localize asymmetrically to primary cilia in sister cell pairs. These results suggest that the segregation of differently aged mother centrioles, an asymmetry inherent to every cell division, might influence how sister cells respond to environmental signals after mitosis, leading to altered behavior or fate for one or both sister cells.

## **(2) Shaping Up and Shipping Out: The Role of Cilia in Growth and Patterning**

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Almost every cell in the body is equipped with a specialized structure called the primary cilium. In addition to their well described roles in mechanosensation, emerging evidence indicated that cilia also participate in intercellular signaling. In this capacity, cilia have been equated to cellular antennae that detect molecular signals in the environment and this influence how cells behave as a consequence. Ciliary effects are associated with a number of human diseases and disorders, the majority of which may be due to the mechanosensory role of cilia. Here we describe a new role for cilia in patterning growth of the craniofacial skeleton.

We disrupted anterograde intraflagellar transport in neural crest cells by inactivating one of the kinesin-II motor subunits, Kif3a in this tissue. The facial prominences and their associated skeletal elements are derived from cranial neural crest: thus, this genetic approach inactivated Kif3a cells that give rise to the facial skeleton. Despite the dramatic truncation in cilia, cranial neural crest cells still migrated normally into the facial prominences. In addition, cranial neural crest cells that lacked cilia still differentiated into osteoblasts and deposited a mineralized matrix on a timescale equivalent to their wild-type and heterozygous littermates. Instead, loss of Kif3a resulted in a dramatic alteration in the pattern and subsequent growth of the facial skeleton. We will discuss the molecular and cellular basis for this defect in skeletal patterning, and present a novel mechanism whereby disruptions in ciliary function are responsible for a class of craniofacial dysmorphologies.

## **(3) Bone Anabolism Achieved By Reducing Sclerostin Bioavailability with an Anti-sclerostin Antibody.**

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While it has been known for decades that bone is a mechanosensitive organ, the responsible mechanism has been elusive. Primary cilia, once commonly thought to be vestigial organelles, are now being re-examined in development and in kidney tissue as a fluid sensing organelle. We have found primary cilia in both osteoblastic and osteocytic cell lines as well as in osteoblasts and osteocytes in vivo [1].

In kidney cells, primary cilia have been established as a necessary component of the cell's fluid flow induced calcium response through stretch activated channels at the base of the primary cilia [2,3]. Calcium mobilization also occurs following fluid shear stress in bone cells [4]. To determine whether primary cilia act as mechanosensors in bone, we examined two outcome variables, intracellular calcium release and prostogandin E<sub>2</sub> release, which have been shown to be independently stimulated by fluid flow [4, 5]. We hypothesized that there would be a correlation between calcium mobilization and the presence of a primary cilium. Since gap junctional communication can transmit calcium signals from cell-to-cell, we also hypothesized that this correlation would increase if gap junctional communication was blocked.

Interestingly while the primary cilium was necessary for the flow induced PGE<sub>2</sub> release, it did not play a role in the cell's mobilization of intracellular calcium. There was also no correlation between whether a cell had a primary cilium, and its likelihood of exhibiting a calcium response. This correlation was not strengthened with the inhibition of intercellular communication by blocking gap junctions. Our findings suggest that primary cilia are a component of the mechanotransduction pathway for PGE<sub>2</sub> release, but distinct from the kidney, do not play a role in intracellular calcium mobilization. This independence of calcium and PGE<sub>2</sub> has been reported

previously and suggests that there are multiple mechanisms by which bone cells sense their mechanical environment. These findings are currently in press in the Proceedings of the National Academy of Science.

- [1] Malone AM. Et al. *ORS No.* 0369, 2006.
- [2] Praetorius HA. Spring KR. *J Membrane Biol.* 191(1):69-76, 2003.
- [3] Praetorius HA. Spring KR. *J Membrane Biol.* 184(1):71-9, 2001.
- [4] You J. et al. *J Biolog Chem.* 276(16):13365-71, 2001.
- [5] Sanders MM et al. *Bone.* 32(4):350-6, 2003.

#### **(4) Intraflagellar Transport in Skeletal Development.**

R Serra

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Primary cilia are non-motile microtubule based appendages extending from the surface of almost all vertebrate cells. The process of Intraflagellar Transport (IFT) is responsible for building and maintaining the structure and function of primary cilia. Disruption of *Kif3a*, a component of the Kinesin-II motor complex, disables anterograde IFT and leads to failure in the formation and maintenance of cilia. Likewise, the absence of *IFT88/Tg737/Polaris*, a core component of the IFT particle, results in the loss of cilia. The functional significance of primary cilia has only recently been uncovered. We recently used the Cre-Lox strategy to target deletion of IFT proteins to skeletal tissue to determine the role of primary cilia in embryonic and post-natal skeletal development (Haycraft et al., 2007; Koyama et al., 2007; Song et al., 2007).

Deletion of *Kif3a* or *IFT88* in *Prx1* expressing cells results in defects in embryonic endochondral bone formation including a dramatic reduction in bone length and accelerated hypertrophic differentiation as early as E14.5 days (Haycraft et al., 2007). *Prx1-Cre* is expressed in early limb mesenchyme starting at E9.5 days and thus recombination is present in chondrocytes and perichondrium/periosteum (Logan et al., 2002). Defects in endochondral bone formation were at least in part due to disruption of Indian Hedgehog (*Ihh*) signaling as measured by the expression of *Ptc1* and *Gli1*, direct targets of Hh signaling. Ectopic cartilage was found in the periosteum along the diaphysis in E18.5 day embryos. This phenotype is distinct from that seen in *Ihh*-null mice and suggests that additional signaling pathways, for example, canonical Wnt signaling, may also be affected by the loss of IFT.

In contrast, deletion of *Kif3a* or *IFT88* in *Col2a* expressing cells did not affect embryonic skeletal development; however, defects in the post-natal growth plate were seen starting at about P7 days (Song et al., 2007). The differences in the two mouse models are likely due to the localization and timing of Cre-mediated recombination. *Col2a-Cre* is expressed later in the limbs (E12.5 days), after cells have committed to the chondrocyte lineage, and the perichondrium/periosteum is not efficiently targeted (Baffi et al., 2004; Ovchinnikov et al., 2000). Even though alterations in skeletal development were not observed until P7 days, *Ptc1* expression was dramatically reduced in chondrocytes as early as E15.5 days. *Ptc1* was maintained in the perichondrium. There is significant evidence for *Ihh* signaling in the perichondrium during embryonic development (Alvarez et al., 2002; Long et al., 2001; Vortkamp et al., 1996) and this may in part explain the lack of an embryonic phenotype in *Col2aCre;Kif3a<sup>f/f</sup>* mice. In the post-natal growth plate, disruption of IFT resulted in reduced proliferation and altered hypertrophic differentiation. Cell shape and columnar orientation in the growth plate were also disrupted suggesting a defect in the process of rotation. Alterations in chondrocyte rotation were accompanied by disruption of the actin cytoskeleton and alterations in the localization of activated FAK to focal adhesion-like structures on chondrocytes. Alterations in Hh signaling as measured by *Ptc1* expression were not detected in the post-natal

growth plate even though the alterations in part resembled those seen in mice with a post-natal disruption of *Ihh* (Maeda et al., 2007). The results suggest that additional signaling pathways may be involved. For example, imaging studies have suggested that chondrocyte cilia could transmit mechanical force through their interaction with the surrounding ECM thus acting as mechanosensors (Jensen et al., 2004).

Alterations were also detected in the cranial base synchondroses in post-natal *Col2aCre;Kif3a<sup>fl/fl</sup>* mice, including growth retardation, disorganization of the growth plate and alterations in chondrocyte differentiation (Koyama et al., 2007). *Ptc1* expression was dramatically reduced in growth plate chondrocytes but the expression domain of *Ptc1* was expanded in the perichondrium suggesting increased Hh signaling in this tissue. Excessive intramembranous ossification as well as ectopic cartilage was seen in perichondrial tissue. Cranial base defects in mice deficient in *Ihh* only minimally resembled those in which *Kif3a* was deleted suggesting that cilia affect signaling through additional factors.

Together, the results indicate that primary cilia/ IFT are involved in multiple signaling pathways within the skeleton.

#### References:

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