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G. Cell Structure and Dynamics

G-1

LRRC6 Regulates the Transcription of Axonemal Components of Motile Cilia

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LRRC6 mutation causes primary ciliary dyskinesia, a disorder resulting from motile cilia defects. However, little is known about specific function of LRRC6 protein at the molecular level. Through transmission electron microscopy of mouse tracheal tissues, we observed that abundancy and morphology were not different between *LRRC6* KO and control littermate mice, but outer and inner dynein arms were absent in motile cilia of *LRRC6* KO mice. Immunofluorescence studies using tracheal tissues and basal cell organoids showed that dynein arm proteins were mainly observed in the cytoplasm, but not in ciliary axonemes in *LRRC6* KOmice. Immunoblot using testis lysates showed the abundance of intermediate chain and heavy chain proteins were significantly decreased in *LRRC6* KO mice compared to control mice. Furthermore RNA-seq data also showed that mRNA levels of intermediate and heavy chain proteins as well as proteins involved in dynein arm assembly were reduced in *LRRC6* KO mice.So far, LRRC6 is known to encode a cytoplasmic protein and is expected to play a role in the assembly of dynein arms. However, given that LRRC6 has a nucleus localization sequence and that mRNA transcripts of dynein subunits are significantly decreased in *LRRC6* KO mice, it seems that LRRC6 translocates to the nucleus and participates in the transcription of dynein arm components during the development of respiratory epithelia.

G-2

Electrically conductive and tailored patterned membrane as regenerative bone and periosteum: thin membrane-modified electrospinning for harnessing topography of 3D printed scaffold

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Traumatic tissue defects are usually accompanied by a loss of tissue membrane that has an impact on the blood supply of organ and has a major role in tissue reconstruction. The absence of periosteum which has important functions of the arrangement of collagen and controlling cell fate might compromise the progress of bone repair, resulting in prolonged recovery time and increased risk of nonunion. Many researchers have studied biomimetic artificial bone, but there are not a lot of researches on how artificial periosteum membrane (APM) surrounding artificial bone plays a role in bone regeneration. In addition, there are few studies that accurately simulate cells and topography in APM. Harnessing topographical cues can provide a non-invasive tool for studying cellular mechanotransduction and expression patterns of protein or gene, through effects on cell morphology. In this study, a developed custom patterned fibrous APM showed a superior capacity in osteogenesis and cell infiltration for accelerating mature bone formation in rat calvarial defects and implied promising strategies for the development of advanced APM with superior bone reconstructive properties.

G-3

Centriole separation and amplification in the *TP53*-, *PCNT*- and *CEP215*-knockout cells

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Centrosome is a subcellular organelle that acts as a microtubule organizing center in an animal cell. In non-dividing cells, primary cilia are derived from the centrosomes. Centrosome number is tightly controlled during the cell cycle. Otherwise, chromosomes are not properly segregated during mitosis, ending up aneuploidy. In this work, we generated *TP53*, *PCNT* and *CEP215* triple knockout (KO) cell line and determined their phenotypes at the centrosome. We observed that the number of centrioles in the triple KO cells is augmented during M phase and maintained throughout interphase However, nascent centriole assembly is significantly limited at S phase. It is interesting that only two out of multiple centrioles are CEP152-positive in the triple KO cells. These results strongly suggest that only a pair of the CEP152-positive centrioles are able to convert into intact mother centrioles which they function as microtubule organizing centers and as templates for centriole assembly during S phase. Nonetheless, we do not rule out the possibility that a fraction of the M-phase-assembled centrioles can function as microtubule organizer at M phase and induce aneuploidy.

G-4

Adaptive Remodeling of Stress Fiber Subtypes in Cyclically Stretched Alveolar Epithelial Cells

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Cyclic stretch applied to cells induces the reorganization of stress fibers. However, the correlation between the reorganization of stress fiber subtypes and strain-dependent responses of the cytoplasm and nucleus has remained unclear. Here, we investigated the dynamic involvement of stress fiber subtypes in the orientation and elongation of cyclically stretched epithelial cells. We applied uniaxial cyclic stretches at 5%, 10%, and 15% strains to cells followed by the release of the mechanical stretch. Dorsal stress fibers and transverse arcs rapidly responded within 15 min regardless of the strain magnitude to facilitate the subsequent changes in the orientation and elongation of the cytoplasm. The cyclic stretched induced the additional formation of perinuclear cap fibers and their increased number was almost maintained with a slight decline after 2-hour-long stretch release. These findings allowed us to propose a model for stretch-induced responses of the cytoplasm and nucleus in epithelial cells based on different mechanoadative properties of stress fiber subtypes.