INTRODUCTION: Osteoporosis is a debilitating bone loss disease which occurs in part when mesenchymal stem cells (MSCs) fail to proliferate in sufficient numbers and/or differentiate down the osteogenic lineage resulting in an imbalance in the bone formation/resorption cycle. A key regulator of MSC proliferation/differentiation is physical loading. The conversion of physical forces into a biochemical response (mechanotransduction) contributes to numerous developmental, physiological and pathological processes particularly in bone [1], yet to date, the mechanotransduction mechanisms in bone marrow derived MSCs remain poorly understood.

The primary cilium is a solitary, immotile, microtubule based cellular extension that protrudes from the apical surface of nearly every cell in the human body. Recent studies have revealed the primary cilium to be a multifunctional antenna, sensing both mechanical and chemical changes in the extracellular environment. In particular, cilia have been shown to act as fluid flow sensors in tissues such as kidney, liver and bone where fluid flow deflects the cilium, triggering intracellular signaling cascades [2]. Furthermore, cilia have recently been shown to act as a pivotal switch guiding biochemically induced lineage commitment in hMSCs. However their role in stem cell mecanotransduction is unknown. In this study we aimed to investigate the mechanosensory role, if any, of the primary cilium in fluid flow mediated changes in proliferation and osteogenic differentiation of hMSCs.

MATERIALS AND METHODS: hMSCs were obtained from two sources (Lonza and STEMCELL) and were maintained in DMEM low glucose media supplemented with 10% FBS and 1% P/S. To visualize primary cilia, hMSCs were cultured in DMEM low glucose containing 0.5% FBS for 2 days. Cells were then fixed and stained for acetylated alpha tubulin which localizes to the cilium (Abcam), phalloidin which stains actin (Invitrogen) and DAPI which stains the nucleus (Invitrogen). The microtubule network within live hMSCs was visualized using Tubulin Tracker (Invitrogen). All cells were imaged using a Leica TCS SP5 Confocal Laser Scanning microscope fitted with a 100x (NA1.46) oil immersion objective. To prevent cilia formation, hMSCs were treated with siRNA (Invitrogen) targeting Polaris (motor protein required for ciliogenesis) for 8hrs using Lipofectamine 2000. Scrambled siRNA was transfected as a control.

72hrs following transfection, hMSCs were subjected to 2hrs of oscillatory fluid flow (OFF) in a parallel plate flow chamber at 1hz frequency at a peak shear stress of 2Pa for proliferation analysis and 1Pa for gene expression analysis. Control cells were placed in identical chambers under static conditions. Upon cessation of flow, slides were placed in fresh media. Proliferation rates were analyzed using EdU staining for primary cilia (red) and actin (green). B/C Microtubule network in (B) XY plane demonstrating the connection of the cilium base to the MT network and (C) YZ plane illustrating cilia orientation in live cell. Arrows indicate cilia.

RESULTS: Primary cilia were visualized as rod like structures extending linearly from the perinuclear region of the cell and were found on 71±6% of hMSCs extending 3-6μm in length. Microtubule (MT) imaging revealed the cilium to be heavily linked with the MT cytoskeleton, projecting out into the extracellular space (Fig.1).

Following transfection with siRNA targeting Polaris, Polaris mRNA expression was significantly reduced by 70% (fig.2) which corresponded to a 62% reduction in number of ciliated cells (data not shown). In cells treated with scrambled siRNA, exposure to flow resulted in a significant 2-fold increase in the proliferation rate of hMSCs, whereas in cells treated with siRNA targeting Polaris, exposure to flow resulted in an even greater 3-fold increase in proliferation rate (fig.2). Regarding osteogenic gene expression, cells treated with scrambled siRNA, exposure to flow resulted in a significant 3.5-fold increase in COX-2 and BMP-2 mRNA expression whereas in cells treated with siRNA targeting Polaris, exposure to flow did not result in a significant change in mRNA expression of either gene (fig.3).

DISCUSSION: In this study we present evidence that the primary cilium exist in high numbers, orientate out into the extracellular space and play a mechanosensory role in hMSCs, providing a novel mechanism by which mechanical stimuli translates into osteogenic responses in this cell type.

Inhibiting primary cilia formation and function significantly increased flow mediated hMSC proliferation rates. Activation of the mTOR pathway is known to enhance cell proliferation and recently the cilium, through the ciliary protein PC1, has been shown to inhibit this pathway. Therefore it has been proposed that cells which do not possess a cilium, cannot control this pathway and thus are hyper-sensitized to a pro-proliferative stimuli such as fluid flow. Our data supports this model and therefore has far reaching significance as many ciliopathies such as polycystic kidney disease are characterized by uncontrolled proliferation and cyst formation. Furthermore, inhibition of cilia formation prevented flow mediated increases in the osteogenic genes COX-2 and BMP-2, both of which are known to be critical for osteoblastogenesis.

These data point to a pro-osteogenic mechanosensory role for the primary cilium in hMSCs, and therefore highlights the cilium as a potential therapeutic target for efforts to prevent bone loss during diseases such as osteoporosis.

SIGNIFICANCE: This study demonstrates a novel mechanotransduction mechanism in human MSCs; highlighting the primary cilium as potential therapeutic target aimed at preventing diseases such as osteoporosis.

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