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ONCOSTREAMS: NOVEL DYNAMICS PATHOLOGICAL MULTICELLULAR STRUCTURES INVOLVED IN GLIOBLATOMA GROWTH AND INVASION

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OBJECTIVES/SPECIFIC AIMS: Oncostreams represent a novel growth pattern of GBM. In this study we uncovered the cellular and molecular mechanism that regulates the oncostreams function in GBM growth and invasion. METHODS/STUDY POPULATION: We studied oncostreams organization and function using genetically engineered mouse gliomas models (GEMM), mouse primary patient derived GBM model and human glioma biopsies. We evaluated the molecular landscape of oncostreams by laser capture microdissection (LCM) followed by RNA-Sequencing and bioinformatics analysis. RESULTS/ANTICIPATED RESULTS: Oncostreams are multicellular structures of 10-20 cells wide and 2-400 μm long. They are distributed throughout the tumors in mouse and human GBM. Oncostreams are heterogeneous structures positive for GFAP, Nestin, Olig2 and Iba1 cells and negative for Neurofilament. Using GEMM we found a negative correlation between oncostream density and animal survival. Moreover, examination of patient's glioma biopsies evidenced that oncostreams are present in high grade but no in low grade gliomas. This suggests that oncostreams may play a role in tumor malignancy. Our data also indicated that oncostreams aid local invasion of normal brain. Transcriptome analysis of oncostreams revealed 43 differentially expressed (DE) genes. Functional enrichment analysis of DE genes showed that "collagen catabolic processes", "positive regulation of cell migration", and "extracellular matrix organization" were the most over-represented GO biological process. Network analysis indicated that Col1a1, ACTA2, MMP9 and MMP10 are primary target genes. These genes were also overexpressed in more malignant tumors (WT-IDH) compared to the less malignant (IDH1- R132H) tumors. Confocal time lapse imagining of 3D tumor slices demonstrated that oncostreams display a collective motion pattern within gliomas that has not been seen before. DISCUSSION/SIGNIFICANCE OF IMPACT: In summary, oncostreams are anatomically and molecularly distinctive, regulate glioma growth and invasion, display collective motion and are regulated by the extracellular matrix. We propose oncostreams as novel pathological markers valuable for diagnosis, prognosis and designing therapeutics for GBM patients.

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Osteocyte-derived CXCL12 is Essential for Load-Induced Bone Formation in Adult Mice

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OBJECTIVES/SPECIFIC AIMS: Our aim is to test whether osteocyte-specific CXCL12 expression is critical to exercise-driven bone formation. METHODS/STUDY POPULATION: All procedures were approved by the NEW YORK UNIVERSITY Institutional Animal Care and Use Committee. We generated male and female mice in which CXCL12 was deleted from OCYs (CXCL12ΔOCY) by crossing CXCL12 floxed mice and 10kb DMP1-Cre transgenic mice (gifts from Drs. Geoffrey Gurtner and Lynda Bonewald, respectively). The 10kb DMP1-Cre has been shown to be robustly expressed in odontoblasts and OCYs, with little to no activity in cells from non-mineralized tissues (Lu+ J Dent Res 2007). Growing male and female mice (n=3-8/group) were given fluorochrome labels every two weeks between 4-16 weeks of age, to monitor the role of CXCL12 during development. A second group, of adult 16-week-old mice (n=5/group), were subjected to tibial axial cyclic loading (1200με, 2Hz, 120cycles, 3days/wk for 2 wks) (Liu+ Bone 2018). Basal and load-induced periosteal (Ps) and endosteal (Es) mineralizing surface (MS/BS, %), mineral apposition (MAR, μm/day) and bone formation rates (BFR/BS, μm3/μm2/year) were calculated (Dempster+ JBMR.2013) at mid-length. RESULTS/ANTICIPATED RESULTS: No significant differences were detected in basal bone formation during development. However, relative load-induced Ps MAR (rMAR) was reduced by 50% in female (p=0.02) and 75% in male (p=0.002) CXCL12ΔOCY mice; and similarly, Ps rBFR/BS was reduced by 50% in female (p=0.01) and 70% in male (p=0.001) CXCL12ΔOCY mice (Figure 1). Es bone formation was not affected by CXCL12 deletion. DISCUSSION/SIGNIFICANCE OF IMPACT: In summary, osteocyte-specific CXCL12 expression plays a critical role in exercise-driven periosteal new bone formation, suggesting that CXCL12 signaling may positively regulate osteogenic differentiation and/or mature osteoblast function. Further underlying mechanisms are currently being explored. Thus, osteocyte-specific CXCL12 signaling may be a promising target to enhance load-induced bone formation in patients with compromised ability to form new bone.

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Overexpression of CD44 is involved in the development of the early endometriotic lesion in a xenograft model

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OBJECTIVES/SPECIFIC AIMS: Previously, we showed decreased development of endometriotic lesions in CD44 knockout mice compared to control.(1) CD44 has 10 different variants and a standard form. Menstrual endometrial cells (MECs) from women with endometriosis have increased adhesion and also express higher levels of CD44 variant 6 (v6) than v3, compared to MECs from women without endometriosis. (2) Here, we assessed the effects of CD44 standard (CD44s), CD44v3 and CD44v6 overexpression (OE) on immortalized human endometrial epithelial (iEECs) and stroma cells (hESCs) in vivo attachment in a nude mouse xenograft model. 1. Knudtson JF, Tekmal RR, Santos MT, et al. Impaired Development of Early Endometriotic Lesions in CD44 Knockout Mice. Reproductive sciences (Thousand Oaks, Calif.). 2016;23(1):87-91. 2. Griffith JS, Liu YG, Tekmal RR, Binkley PA, Holden AE, Schenken RS. Menstrual endometrial cells from women with endometriosis demonstrate increased adherence to peritoneal cells and increased expression of CD44 splice variants. Fertility and sterility. 2010;93(6):1745-1749. METHODS/STUDY POPULATION: Overexpression of CD44s, CD44v3 and CD44v6 was carried out using lipofectamine and their expression verified with qRT-PCR in iEEC and hESCs. Nude mice, 8-10 week old, were injected with estrogen 1 week prior to injection of iEECs and hESCs (n=7 per group). The cells were counted after transfection and at least 300,000 iEECs and 300,000 hESCs were injected per mouse. The transfected cells were tagged with cell tracker red (iEECs) and green (hESCs). Forty-eight hours after injection into the

https://doi.org/10.1017/cts.2019.253 Published online by Cambridge University Press