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Dural Cells Release Factors Which Promote Cancer Cell Malignancy and Induce Immunosuppressive Markers in Bone Marrow Myeloid Cells

BACKGROUND: Thirty per cent of cancer patients develop spine metastases with a substantial number leading to spinal cord compression and neurological deficits. Many demonstrate a propensity toward metastasis to the posterior third of the vertebral body. The dura, the outer layer of the meninges, lies in intimate contact with the posterior border of the vertebral body and has been shown to influence adjacent bone. The effects of the dura on bone marrow and cancer cells have not been examined. Understanding the biology of spinal metastasis will provide insights into mechanisms of cancer growth and allow for new treatment strategies.

OBJECTIVE: To examine the extent to which dura influences bone marrow/tumor cell metastatic characteristics.

METHODS: Dura conditioned media (DCM) from primary dura was examined for the ability to stimulate tumor cell proliferation/invasion and to alter bone marrow cell populations. RNA sequencing of dural fibroblasts was performed to examine expression of cytokines and growth factors.

RESULTS: DCM induced a significant increase in invasion and proliferation of multiple tumor cell lines, and of patient-derived primary spinal metastatic cells. DCM also increased the proliferation of bone marrow myeloid cells, inducing expression of immunosuppressive markers. RNA sequencing of dural fibroblasts demonstrated abundant expression of cytokines and growth factors involved in cancer/immune pathways.

CONCLUSION: Factors released by primary dural cells induce proliferation of tumor cells and alter bone marrow to create a fertile environment for tumor growth. The dura therefore may play an important role in the increased incidence of metastases to adjacent bone.

KEY WORDS: Bone marrow, Cytokines, Dura mater, Fibroblasts, Metastatic disease, Spinal cord compression

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Nearly 300 000 adults in the United States currently have osseous metastatic disease, the spine being the most common site with over 50% of bone metastases found in the spinal column in some

histologies (Figure 1).¹⁻³ Approximately 10% of patients with spine metastasis will develop spinal cord compression, a severe and often permanently disabling condition that is a true oncologic emergency. Beyond spinal cord compression, spine metastases frequently lead to significant morbidity including severe pain, radiculopathy from nerve root compression, pathological fractures, and hypercalcemia.⁴ The incidence of spinal metastatic disease has been increasing over the past 20 years, likely due to (1) improvements and increased utilization of MRI that allows for earlier diagnosis; (2) an increasing elderly population, providing a larger pool of patients who may develop cancer and subsequent spinal metastases; and (3)

ABBREVIATIONS: BM, bone marrow; CNS, central nervous system; DCM, dura conditioned media; GF, growth factor; HCC, hepatocellular carcinoma; H&E, hematoxylin and eosin; KEGG, Kyoto Encyclopedia of Genes and Genomes; MDSC, myeloid-derived suppressor cells; RCC, renal cell carcinoma; RS, renal sarcoma; RTK, receptor tyrosine kinases; TME, tumor microenvironment

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advances in the systemic treatment of cancer allowing for longer survival, thus increasing the odds of developing spinal metastatic disease.

Not only is the spine one of the most common sites of metastatic disease, the posterior third of the vertebral body has a higher concentration of metastasis.^{1,5} A key feature unique to the spinal skeleton is represented by the presence of the dura mater, the covering of the central nervous system (CNS), located in intimate contact with the posterior aspect of the vertebral body (Figures 2A and 2B).

The dura mater is the outer layer of the meninges that forms a protective tube around the spinal cord inside the vertebral canal. It is a dense, collagenous connective tissue made of different cell types involved in cancer modulation, including fibroblasts, osteoprogenitor cells, and blood vessels.^{6,7} Reciprocal interactions

between the dura and the surrounding tumor microenvironment (TME) by both autocrine and paracrine signaling have been reported.⁸⁻¹¹ During development, dura mater regulates morphogenesis in the CNS and the overlying bone by actively modulating the surrounding environment, secreting factors such as CXCL12, FGF2, and TGF β that guide proliferation, migration, and differentiation of neural precursor cells.¹² The increased predilection of cancer metastases to the spine and the unique presence of the dura in the spinal microenvironment led us to hypothesize that the dura affects tumor cell growth directly by secreting factors that act on tumor cells and/or indirectly by altering the phenotype of cells in the spinal metastatic niche.

To date, no studies have explored the role that the dura may play in promoting tumor metastasis. The purpose of this investigation was to explore the impact of the dura on the specific biology of metastases. This work demonstrates for the first time that dura-derived cells produce secreted factors that promote tumor growth through direct activation of tumor cells, and modulate the bone marrow (BM) microenvironment to enhance tumor growth in bone.

METHODS

Hematoxylin and eosin (H&E) staining and immunohistochemistry were performed using standard techniques (see **Supplementary Methods, Supplemental Digital Content 1** for full details).

Generation of Dural Fibroblasts

Dura was collected from the calvaria of 4 to 6-wk-old C57BL/6/J mice, minced, and cultured in Dulbecco Modified Eagle Medium (DMEM)/Fetal Bovine Serum (FBS) for 3 to 4 passages before dural fibroblasts were used for experiments and to generate dura conditioned media (DCM). Further methods details are presented in the **Supplementary Methods, Supplemental Digital Content 1**.

Generation of Patient-Derived Primary Spinal Metastatic Cells

Tumor samples were collected during spinal surgeries performed to remove the tumor and stabilize the spine in accordance with the university approved Institutional Review Board (IRB) protocol. Cells were generated from spinal metastases from primary renal cell carcinoma (RCC), renal sarcoma (RS), and hepatocellular carcinoma (HCC). Spinal tumors were minced into small fragments, digested in 1.25% collagenase (# 17104019, ThermoFisher Waltham, Massachusetts), filtered into single cell suspensions, and cultured in DMEM/FBS at 37°C, 5% CO₂. Cells were passaged 1:2 at 80% confluence for 3 to 6 passages and then used for experiments or cryopreserved.

Western Blot Analysis and Immunohistochemistry

DU145 prostate carcinoma cells were deprived of serum for 15 h and treated with either DMEM/1% Bovine serum albumin (BSA) (control) or DCM: DMEM/1% BSA (25%) and serum-free DCM (75%) for 45 min and 15 h. Western blot analysis and immunohistochemistry were performed using standard methods. Antibodies used are reported in the **Supplementary Methods, Supplemental Digital Content 1**.

FIGURE 2. The dura is in close apposition to the vertebral bodies, communicating directly with the spinal bone marrow at the level of the vertebral foramina. When dissociated and cultured, these cells have characteristics of fibroblasts. **A**, Low magnification ($\times 10$) micrograph showing a sagittal section through a thoracic mouse vertebra and adjacent spinal cord. Note the direct contact of the dura with both the vertebral bone and spinal cord. **B**, High magnification ($\times 20$) micrograph of a different area showing direct contact of the dura with the spinal bone marrow at the level of the vertebral foramen. These foramina are numerous in the vertebral body cortex and can also be seen with veins or arteries traversing this space. Scale bars in **A** and **B** equal 200 μm and 50 μm . **C**, Immunohistochemistry ($\times 20$) Vimentin stain of dissociated dural cells showing morphological features of fibroblasts as well as positive vimentin staining (brown).

Proliferation and Fluorescence-activated cell sorting (FACS) Analysis of BM Cells

BM cells were collected from the thoracic and lumbar vertebrae of 4 to 6-wk-old C57BL6/J mice, red blood cells were lysed, and mononuclear cells were used for proliferation analysis in control media (Roswell Park Memorial Institute (RPMI)/10% FBS) or DCM culture medium: RPMI/10% FBS (50%) and serum DCM (50%). Cells were analyzed at indicated times using the CellTiter-Glo[®] Luminescent Cell Viability Assay (Promega, Madison, Wisconsin).

Spine BM cells were collected as described above, and cultured in either control or DCM medium, which was replenished every 48 h. After 5 d, cells were collected, immunostained, and analyzed on a BD-FACS ARIA III (Becton Dickinson, Franklin Lakes, New Jersey), for expression of myeloid cell markers. Antibodies used are presented in the **Supplementary Methods, Supplemental Digital Content 1**. Data were analyzed using FlowJo_V10 (FloJo LLC, Ashland, Oregon). Graphical representation and statistical analysis were

performed with GraphPad Prism V7.00 (GraphPad Software, La Jolla, California)

Quantitative RT-PCR

QRT-PCR of mouse and human dura was performed using standard methods (details in **Supplementary Methods, Supplemental Digital Content 1**). In brief, RNA was extracted from either mouse dura fibroblasts or from flash-frozen human dura obtained from autopsy specimens, and analyzed for transcript abundance. Expression was calculated and represented graphically as fold change compared to control.

Quantitative RT-PCR of CD11b⁺ cells cultured in control or DCM

BM cells were collected and immunostained with antibodies for CD45 and CD11b. CD45⁺CD11b⁺ cells were sorted using a BD-FACS ARIA III and cultured in control or DCM medium. After 32 h,

cells were collected, RNA extracted, converted into cDNA (#4368814, ThermoFisher). QRT-PCR was performed using gene-specific primers as described above. Sequences of the mouse and human primers used are presented in the **Supplementary Methods, Supplemental Digital Content 1**. Methodological details for RNA sequencing and analysis are presented in the **Supplementary Methods, Supplemental Digital Content 1**.

RESULTS

Secreted Factors From the Dura Promote Proliferation and Invasion of Spinal Metastatic Cells, Activating Oncogenic Signaling Pathways

We hypothesized that the unique arrangement of the dura in close proximity to the spine is a critical factor responsible for the high incidence of spinal metastases. To test the extent

to which secreted factors from the dura promote tumor growth and invasion, we harvested and expanded murine dural cells and collected DCM. Analysis of these cultured cells by immunohistochemistry shows that dural-derived cells express high levels of vimentin, validating the fibroblast identity of these cells. Also, morphological features of dural cells in culture were consistent with fibroblasts (Figure 2C). Technical details pertaining to results shown in Figure 2 are presented in the Methods section: H&E staining and immunohistochemistry. Proliferation analysis of several prostate (DU145, C42B, and PC3) and breast carcinoma (MDA-MB231) cell lines showed that DCM increases proliferation and survival of the DU145 and MDA-MB-231 carcinoma cells, but not of the C42B or PC3 cells after 24 and 48 h in culture (Figure 3A). These results may reflect intrinsic differences in the cells or the microenvironment from which they were obtained.

We then examined the effects of DCM on human primary cells derived from surgically obtained spinal metastases originating from RCC, RS, and HCC specimens. Early passage cells (passages 3–6) were grown in low serum conditions with or without DCM, and proliferation was analyzed over the course of 3 d. DCM promoted proliferation of all primary cell cultures derived from spine metastases after prolonged exposure (48 h and 72 h; Figure 3B). These data suggest that factors secreted from the dura create a favorable environment for spinal metastatic growth.

Local tissue remodeling is critical for enabling tumor growth and invasion. Numerous studies support the role of cancer-associated fibroblasts in tissue remodeling and in promoting the invasive phenotype of tumor cells.^{13,14} To test the extent to which secreted factors from dura influence the invasive potential of carcinoma cells, we performed invasion assays by assessing the number of cells that traversed an artificial extracellular matrix in response to DCM. DCM promoted significant invasion of DU145, PC3 prostate, and MDA-MB-231 breast carcinoma cell lines (Figure 3C).

Several pathways activated by extracellular signaling molecules acting on many receptor tyrosine kinases contribute to the growth, migration, and invasion of tumor cells, including the PI3K/AKT,^{15–18} RAS/RAF/MEK/ERK^{15–17,19,20} and STAT3.²¹ DCM induces activation of signaling proteins in all of these 3 pathways tested, inducing a rapid and prolonged activation of pAKT, pERK1/2, and pSTAT3 (Figure 3D). Methodological details pertaining to experiments presented in Figure 3 are presented in the Methods and **Supplementary Methods, Supplemental Digital Content 1** sections: generation of dural fibroblasts, generation of patient-derived primary spinal metastatic cells, generation of dural conditioned media, and analysis of tumor proliferation and invasion. These data suggest that the dura represents one of the factors in the spinal TME, promoting tumor growth and invasion.

BM Myeloid Cells Show Increased Survival/Proliferation and Increased Expression of Immunosuppressive Markers When Cultured With DCM

A major presence in the spinal microenvironment is represented by the BM, CD11b⁺ cells representing about half of all BM cells. Immature myeloid cells, termed myeloid-derived suppressor cells (MDSCs), induced to proliferate and migrate into the TME by tumor-derived chemokines, have been described as an important population of cells contributing to tumor-induced immune suppression and immune evasion in numerous cancers.^{22,23} We tested the effect of DCM on the growth and phenotype of BM cells. Proliferation analysis of spine BM cells cultured with DCM demonstrates that DCM induced substantial survival and proliferation of BM cells over the course of 5 d. In the absence of DCM, the majority of BM cells did not survive in culture (Figure 4A). Similar results were seen with BM cells collected from long bones (tibia and femur, data not shown). The composition of the BM cells after the DCM treatment

was analyzed using murine markers of MDSCs (CD11b and Gr1 [Ly6C and Ly6G]). We observed that the majority of cells after 5 d in culture were CD11b⁺: DCM inducing a significant increase in the percentage of CD11b⁺ cells (77.32% vs 54.27%, $P < .001$), CD11b⁺Gr1^{low} (56.21% vs 32.42%, $P < .001$) cells, and CD11b⁺Gr1^{neg} cells (53.92% vs 43.2%, $P < .001$; Figures 4B and 4C). In the mouse, CD11b⁺Gr1^{high} represent granulocytic MDSCs (Ly6C^{high}), whereas the CD11b⁺Gr1^{low} the monocytic MDSCs (Ly6C^{high}).²⁴ We observed similar changes in the proportion of CD11b⁺ subsets in experiments performed with CD11b⁺ BM cells purified by FACS sorting (data not shown). To analyze if secreted factors from the dura induce phenotypic changes in these myeloid cells, we collected RNA from sorted CD11b⁺ cells (Figure 4D) cultured in control or DCM media for 32 h and analyzed expression of functional activation markers by QPCR. DCM induced expression of immunosuppressive markers of activation: *nos1*, *arg-1*, *cd206*, *tgfb1*, and *tgfb1l1*, but did not change the expression of the costimulatory molecule *cd86* (Figure 4E). Technical details for experiments presented in Figure 4 are found in the Methods section: Proliferation and FACS analysis of BM cells and Quantitative RT-PCR of CD11b⁺ cells cultured in control of DCM. Taken together, these data demonstrate that factors from the dura induce proliferation and survival of BM myeloid cells, increase the proportion of monocytic and Gr1^{neg} CD11b cells, and enhance expression of immunosuppressive molecules, known to facilitate tumor growth by promoting tumor immune escape.

Dural Fibroblasts Show an Enriched Molecular Signature for Secreted Factors Driving Cancer Pathways

Thus far, factors produced by dural cells stimulate proliferation of tumor and BM cells. To identify secreted factors specific for the dura, we compared the transcriptome of dural and mouse tail-tip fibroblasts.²⁵ This analysis identified 3827 differentially expressed genes, from a total of 22 860 genes with measured expression ($P < .01$, LogFC > 1). Pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database identified 124 significantly altered pathways between the 2 datasets. The top pathways included pathways in cancer (KEGG 05200, 129 genes), transcriptional mis-regulation in cancer (KEGG 05202, 57 genes), and osteoclast differentiation (KEGG 05200, 45 genes; Figure 5, and **Figure, Supplemental Digital Content 2**). Many of the differentially expressed genes are growth factors (GF) and cytokines. Further analysis of GFs and cytokines with measured expression (122 genes) identified 78 genes with differential expression ($P < .05$, LogFC > .585). Pathway analysis of this subset ranked in the top 5 were pathway in cancer (KEGG 05200, 19 genes), breast cancer (KEGG 05224, 8 genes), and TGFβ signaling (KEGG: 04350, 10 genes). Other pathways in the top 25 included proteoglycans in cancer (KEGG: 05205, 10 genes), melanoma (KEGG: 05218, 8 genes), RCC (KEGG: 05211, 6 genes), and rheumatoid arthritis (KEGG: 05323, 14 genes) (Figures 6A–6G). Hence, KEGG analysis placed secreted

FIGURE 4. Conditioned media from the dura (DCM) promotes survival and proliferation of bone marrow myeloid cells and increases their expression of immunosuppressive markers. **A**, Proliferation analysis of vertebral BM cells cultured in control or DCM containing media over a 5-d time-course. Data points represent averages and SEM of 4 replicate wells. **B**, Representative dot-plot graphics of vertebral BM (5-d culture with or without DCM) immunostained with CD45, CD11b, and Gr1. **C**, Quantitative analysis of BM myeloid populations treated or not for 5 d with DCM, showing increase in the percentage of CD11b⁺ cells ($P < .001$), CD11b⁺Gr1^{low} ($P < .001$), and CD11b⁺Gr1^{neg} myeloid cells. **D**, Frequency of BM populations before (left panel) and after (right panel) FACS sorting, illustrating a high purity (>93%) of the post-sort CD11b⁺ population. **E**, Graphical representation of QPCR analysis showing expression of immunosuppressive markers in sorted CD11b⁺ BM cells immediately after sorting (control 0 h) and after 32 h culture in control or DCM containing media. Data show an increase in arginase 1 ($P = .001$), cd206 ($P < .001$), tgfβ1 ($P = .001$), and tgfβRII ($P = .0129$). Data represent averages and SEM of triplicate sample wells. Two-tailed unpaired *t*-tests were performed for statistical analysis.

factors from the dura within signaling pathways known to promote cancer dissemination and growth. The expression values ($\log_2\text{TPM}$) of this subset of GFs and cytokines were represented graphically as a heatmap, illustrating that fibroblasts from the dura exhibit an increased secretory activity, producing more GFs and cytokines than fibroblasts isolated from mouse tail

tips (Figure 6H and **Figure, Supplemental Digital Content 3**). Methodological details for RNA sequencing and analysis are presented in the **Supplementary Methods, Supplemental Digital Content 1**.

In our experiments we have used mouse calvarial dura to generate fibroblasts, as spinal dura is quantitatively very limited.

To test if secreted cytokines from the calvarial and spinal dura are significantly different, we extracted RNA from human autopsy dura and performed QRT-PCR with human-specific primers for several cytokines. Data show high expression of the CXCR2 ligands CXCL1 and IL8, the CXCR4 ligand CXCL12, and of the macrophage colony stimulating factor CSF1 in both spine and calvarial dura (**Figure, Supplemental Digital Content 4**) suggesting that the spine and calvarial dura produce a similar repertoire of secreted factors.

DISCUSSION

The increasing incidence of spinal metastases brings to the forefront the need to increase our understanding of factors promoting preferential dissemination and growth of metastases to the spine. The identification of contributing factors and their importance may lead to new treatment options. Accumulating evidence highlights the role of the TME in facilitating metastatic dissemination and growth and the need for targeting the TME to improve cancer therapies.²⁶⁻²⁸ Cells of the microenvironment, including fibroblasts and BM cells, coexist in a functional homeostatic environment with self-regulating feedback loops guiding production of secreted factors required to ensure physiological functions. Tumor cells disseminate to and thrive in environments that produce factors facilitating their survival and growth. The key to developing treatment strategies is to identify these factors and the mechanism through which they function.

We show for the first time that the dura, a unique anatomical component of the spinal microenvironment, has the potential to promote the growth of numerous tumor cells and also to skew the BM microenvironment toward an immunosuppressive phenotype, known to facilitate tumor growth and dissemination. We identified by RNA seq a number of secreted factors present in the dura which are known to drive cancer pathways, such as BMP4, FGF2, FGF11, FGF13, IGF1, IGF2, CXCL12, TGF α , TGF β 1, TGF β 2, and TGF β 3, VEGF α , VEGF β , CTGF, and PDGF β , and this profile is different from other RNA fibroblast profiles we compared (Figures 5 and 6; **Figure, Supplemental Digital Content 3**). The question that remains to be answered is whether this unique profile leads to a unique effect on tumor cells.

These secreted factors contribute to the growth, migration, and invasion of tumor cells by activating several signaling pathways. Akt is a serine/threonine signaling kinase signaling through PI3K to promote cellular growth and survival. Large-scale genomic analyses of prostate and breast carcinoma patients^{15,16,18} have shown increased activation of Akt due to increased signaling through receptor tyrosine kinases (RTK) and chemokine receptors and also due to the functional loss of the tumor suppressor phosphatase and tensin homolog (PTEN), a strong negative regulator of Akt. In advanced and metastatic prostate cancer, alterations in the PI3K/Akt pathway are the second most common genomic alterations.¹⁷ Activation of the mitogen-activated protein kinase/extracellular signal-regulated

kinase RAS/RAF/MEK/ERK pathway is central to the pathogenesis of numerous malignancies, associated with poor prognosis and metastatic dissemination.²⁰ Also, activation of STAT3, downstream of numerous RTKs and nonreceptor tyrosine kinases, has important roles in tumor progression promoting tumor cell survival and proliferation, as well as activation of numerous cells in the TME, including fibroblasts and immune cells.²¹ DCM induced all these pathways in a prostate carcinoma cell line derived from a dural metastasis (DU145). DCM induces proliferation and invasion of several carcinoma cell lines and primary spinal metastatic cells, compelling evidence to support the role of the dura in promoting malignancy and contributing to the high incidence of metastasis in the spine. We observe differences in responses to DCM between cell lines, most likely related to differential receptor expression on these cells, or to altered signaling pathways from specific repertoires of genetic alterations. Still to be determined are which of the secreted factors or which combination of secreted factors produced by the dura are the most potent promoters of the observed phenotype and if results from in vitro experiments will be supported by in vivo data in animal models of spinal metastasis.

Recently, a large body of scientific work has identified immunosuppression as a critical factor in the pathogenesis and progression of cancer, a mechanism responsible for the failure of immunotherapies for malignant diseases.^{29,30} In addition to the direct effect of increased growth and invasion on tumor cells, we demonstrate that secreted factors from the dura significantly affected the number and phenotype of BM myeloid cells. Immature myeloid cells, termed MDSCs, induced to proliferate and migrate into the TME by tumor-derived chemokines, have been described as an important population of cells contributing to tumor-induced immune suppression and immune evasion in numerous cancers.^{22,23} These MDSCs are characterized by production of molecules such as ROS factors (superoxide, hydrogen peroxide, and peroxynitrite), arginase-1, nitric oxide synthase 1 (NOS1), and several chemokines such as IL-10 and TGF β that inhibit activation and proliferation of T cells and prevent functional differentiation of antigen-presenting cells.²⁴ The abundant presence of immature myeloid cells in the bone microenvironment may represent a factor contributing to the high incidence of bone metastasis in multiple cancers. Our data show a high abundance in the dura of members of the TGF β and IGF family of secreted factors, known to induce potent immunosuppression and promote immune evasion.³¹⁻³³ Treatment of BM cultures with DCM promotes the survival and proliferation of CD11b myeloid cells and the expansion of CD11b⁺Gr1^{low} and CD11b⁺Gr1^{neg} cells, increasing the expression of immunosuppressive markers, characteristic of MDSCs. Further work is needed to confirm the functional properties of these myeloid cells and identify the specific dural factors responsible for the observed phenotype. The RNA seq data presented provide valuable and compelling candidates to begin targeted experiments to dissect the contribution of specific molecular players in the biology of spinal metastatic disease.

Limitations

As mentioned above, the mouse spinal dura is not readily amenable to experimental exploration due to limited availability, a reason for which we have used mouse calvarial dura in these experiments. We have comparatively analyzed selected cytokines, which we identified at high levels in the mouse calvarial dura, in human postmortem specimens of human cranial and spinal dura (**Figure, Supplemental Digital Content 4**). This analysis shows expression of these cytokines in both spinal and cranial dura, suggesting that dura from both sites are bioactive tissues, with similar secretory patterns. Further studies would be needed to establish significant differences in expression patterns between calvarial and spinal dura. This study represents an initial investigation into the role of dura secreted factors on the osseous microenvironment and tumor cells.

CONCLUSION

This study demonstrates that the dura is a bioactive tissue, composed primarily of fibroblasts, which releases factors that have a direct effect on tumor cell invasion and proliferation, activating downstream signaling pathways associated with tumor progression. These factors also act on myeloid cells in residence in the BM of the spine and cause them to proliferate and express markers that denote immunosuppression. Ongoing work in our laboratory aims to further refine our knowledge of the role of the dura in cancer metastases and progression and to identify targetable molecules amenable for therapeutic intervention, to prevent, and mitigate clinical consequences of spinal metastatic disease.

Disclosures

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Supplemental Digital Content 1. Supplementary Methods.

Supplemental Digital Content 2. Figure. Pathway analysis identified in the top 5 pathways in addition to pathways in cancer, enrichment of genes associated with transcriptional misregulation in cancer and osteoclast differentiation (**B**). **A**, Differential expression of genes associated with transcriptional misregulation in cancer (KEGG 05202, 57 genes). **B**, Differential expression of genes involved in osteoclast differentiation (KEGG 05200, 45 genes). Genes upregulated in dura fibroblasts are represented as red bars.

Supplemental Digital Content 3. Figure. Heatmap of growth factors and cytokines with measured expression in the dura and tail-tip fibroblasts. The left panel illustrates the heatmap of expressed cytokines and the right panel of expressed growth factors in the dura and tail-tip fibroblasts. Secreted factors are ranked based on normalized expression values of transcripts per million (log2TPM) and color coded as illustrated in the color scale bar.

Supplemental Digital Content 4. Figure. Human spine and calvarial dura produce cytokines similar to the mouse calvarial dura. Human spine and calvarial dura were obtained from autopsy samples. RNA was extracted and processed for QRT-PCR with human-specific primers, to measure expression of selected

cytokines, observed to have high expression in the mouse calvarial dura. Data show expression of the CXCR2 ligands CXCL1 and IL8, the CXCR4 ligand CXCL12, and of the macrophage colony stimulating factor CSF1 in both spine and calvarial dura.

COMMENT

These authors present a novel hypothesis that fibroblasts in the dura affect the spine microenvironment, promoting the invasion and proliferation of metastasis. In tissue culture, proliferation and invasion of several carcinoma cell lines was enhanced in dural conditioned media as compared to controls. RNA sequencing of dural fibroblasts showed expression of cytokines and growth factors related to cancer pathways. Some factors affect the adjacent bone marrow myeloid cells, inducing expression of markers associated with immunosuppression, which may allow cancers to proliferate. This paper provides new insights regarding the pathophysiology of spinal metastasis.

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