β2-Microglobulin Induces Epithelial to Mesenchymal Transition and Confers Cancer Lethality and Bone Metastasis in Human Cancer Cells

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Abstract

Bone metastasis is one of the predominant causes of cancer lethality. This study demonstrates for the first time how β2-microglobulin (β2-M) supports lethal metastasis in vivo in human prostate, breast, lung, and renal cancer cells. β2-M mediates this process by activating epithelial to mesenchymal transition (EMT) to promote lethal bone and soft tissue metastases in host mice. β2-M interacts with its receptor, hemochromatosis (HFE) protein, to modulate iron responsive pathways in cancer cells. Inhibition of either β2-M or HFE results in reversion of EMT. These results demonstrate the role of β2-M in cancer metastasis and lethality. Thus, β2-M and its downstream signaling pathways are promising prognostic markers of cancer metastases and novel therapeutic targets for cancer therapy. Cancer Res; 71(7); 1–11. ©2011 AACR.

Introduction

Bone is the second most common site of cancer metastasis, harboring over 70% of cancer metastases from prostate and breast cancers (1). Advanced-stage cancer patients develop bone metastases either with or without hormonal therapy, radiation therapy, chemotherapy, and immunotherapy, and currently there is no effective treatment. The pathogenesis of bone metastases remains poorly understood. So far there is no known transgene which reliably promotes cancer bone metastasis in immune-deficient mice or in immune-competent transgenic animals when expressed in cancer or normal cells. Here we demonstrated that overexpression of β2-microglobulin (β2-M) drives epithelial to mesenchymal transition (EMT) promoting lethal cancer bone and soft tissue metastases in human prostate, breast, lung, and renal cancers in vivo.

β2-M, a 11 kDa nonglycosylated protein, exists in all nucleated cells (2, 3). β2-M is involved in the regulation of the host immune response (4, 5). β2-M was reported by our laboratory (6–8) and others (9–11) as a growth factor and signaling molecule in cancer cells. β2-M expression increases during progression of human prostate cancer (9), breast cancer (12), renal cancer (13), lung cancer (14), colon cancer (15), and a number of liquid tumors (11). β2-M is a pleiotropic signaling molecule regulating protein kinase A androgen receptor, vascular endothelial growth factor (VEGF; ref.7), fatty acid synthase (8), and lipid-raft-mediated growth and survival (11) signaling pathways. β2-M has multiple roles in cancer development and mediates tumorigenesis, angiogenesis, and osteomimicry (7). β2-M is also known to activate stromal cells such as mesenchymal stem cell (16), osteoblasts (17), and osteoclast (18). β2-M interacts can interact with major histocompatibility complex (MHC) class 1, classical, and nonclassical members. One of the nonclassical member is hemochromatosis (HFE) protein. β2-M knockout mice and HFE knockout mice have several identical pathophysiologic phenotypes, and develop symptoms of hemochromatosis involving iron overload and its associated diseases (19, 20). Several studies demonstrate the interaction between β2-M/HFE and its physical interaction with transferrin receptor, the primary mechanism for iron uptake in mammalian cells (21). In the present study, we demonstrated that HFE interacts with β2-M, modulating iron homeostasis, and governs EMT in cancer cells. We identified HFE as a β2-M receptor, which activates iron responsive HIF-1α (hypoxia inducible
factor-1α) signaling pathways and promotes cancer bone and soft tissue metastases.

Materials and Methods

Cell culture

Human androgen-refractory prostate cancer ARCaPE (androgen refractory prostate cancer—epithelial clone) and ARCaPE2 (androgen refractory prostate cancer—mesenchymal clone) and C4–2 prostate cancer [derived in the laboratory (22, 23)], MCF7 breast cancer and H358 nonsmall cell lung cancer cells (from ATCC) were cultured in T-medium (GibcoBRL) supplemented with 5% heat inactivated fetal bovine serum (FBS; Bio-Whittaker). Renal cancer SN12C cells (from ATCC) were cultured in minimum essential medium (MEM; GibcoBRL) with 10% FBS. Each had 50 IU/mL penicillin and 50 μg/mL streptomycin (GibcoBRL) in 5% CO₂ at 37°C. All cells were tested for mycoplasma (Mycoplasma detection kit (R&D systems), and were found to be negative.

Plasmid construction and stable transfection of β2–M expression vector

Mammalian expression plasmid for human β2-M in pcDNA3.1 was described previously (7). Empty pcDNA3.1 expression vector was used as control (Neo). MCF7, H358 and SN12C cells were transfected into plasmid with Lipofectamine 2000 (Invitrogen) and positive stable clones were established. Control and β2-M siRNA was retrovirally transfected into ARCaP2 cells and are indicated as knockdown cells (KDI and KDII).

ELISA

β2-M protein concentration in blood and culture media was assayed by the Quantikine IVD human β2-M ELISA kit (R&D Systems).

Invasion and migration assays

Cancer cell invasion and migration were assayed in Companion 24-well plates (Becton Dickinson Labware) with 8 μm porosity polycarbonate filter membranes as described previously (24).

RNA preparation and reverse transcription (RT)-PCR analysis

Total RNA was isolated from confluent monolayers of cells using the RNeasy Mini Kit (QIAGEN). RT-PCR was performed as previously described (24).

Immunoblot analysis and flow cytometry

Western analysis was performed as previously described (24). The membranes were incubated with mouse monoclonal antibody against β2-M (Santa Cruz Biotechnology), E-cadherin (BD Biosciences), N-cadherin (BD Biosciences), Vimentin (Santa Cruz Biotechnology), HFE (Santa Cruz Biotechnology), and HIF-1α (Millipore) respectively, at 4°C overnight. Intracellular flow cytometric analysis was performed using BD CytoFix to permeabilize the cells followed by primary and secondary antibody treatments.

In vivo animal experiments

All animal experiments were approved and done in accordance with institutional guidelines. Four-week-old male or female athymic nude mice (19–21 g; BALB/c nu/nu mice, NCI) implanted with an 17β-estradiol pellet (NE-121, Innovative Research of America) subcutaneously were injected with 1 × 10⁶ cells suspended in 10 μL sterile PBS into both tibias (n = 8). The estimated volume of bone tumors was calculated by 3 axes (X, Y, and Z) measured from a radiograph using the formula π/6XYZ (25). Tumor size was also quantified by measuring hind limb diameter every 5 days. For intracardiac injection, anesthetized mice were injected with 5 × 10⁵ cells/50 μL PBS/mouse into the left ventricle of the heart by nonsurgical means using a 28G1/2 needle (26). Metastases to distant organs were confirmed by radiography, necropsy, and histomorphology of the tumor specimens.

Immunohistochemistry

IHC was used to determine the level of protein expression in bone specimens. The following primary antibodies were used: E-cadherin (H-108; Santa Cruz Biotechnology) for E-cadherin, N-cadherin antibody (Abcam) for N-cadherin, Vimentin (V9; Santa Cruz Biotechnology) for vimentin, and β-2-Microglobulin (BBM.1; Santa Cruz Biotechnology) for β2-M. IHC staining was performed as previously described (24). Tartrate-resistant acid phosphatase (TRAP) staining was also performed to detect osteoclasts as previously described (24).

Immunoprecipitation

Immunoprecipitation was performed using the immunoprecipitation starter pack (GE Healthcare).

Lentiviral transduction

Lentiviral transduction was performed as per instructions (Sigma). Cells were selected using puromycin (4 μg/mL). Control cells which did not receive the viral particles died in 3 to 5 days. HFE shRNA transduced cells were characterized for HFE levels 7 to 10 days after transduction.

Iron measurements

Iron concentration was determined using induced coupled plasma mass spectroscopy (ICP-MS). Cells were grown to 10⁷ cells and pelleted and digested using 3% nitric acid. Samples were diluted and analyzed by Perkin Elmer ICP-MS. The data are expressed as picomoles of metal.

Iron chelator and hypoxia treatments on ARCaPE

ARCaPE cells were treated with 200 μmol/L of DES (desferal) for 48 hours. Then the DES was removed and replaced with normal media. A day later, cells were photographed and cell lysates were prepared for immunoblot analysis. ARCaPE cells were exposed to hypoxia (1% O₂, 5% CO₂, and remaining N₂) in humidified airtight chambers for 72 hours, cells were photographed and cell lysates were prepared for immunoblot.
Statistical analysis

Values were expressed as means ± standard deviation. Statistical analysis was performed using Student’s t-test or one-way ANOVA. Relationships between qualitative variables were determined using the χ² test. The estimated probability of survival was obtained using Kaplan–Meier methodology and differences were evaluated by log-rank test. Values of $P < 0.05$ were considered to be statistically significant.

Results

β2-M induces increased invasion and migration in breast, lung, and renal cancer cells

Our previous studies showed that ARCaP$_E$ cells, a subclone of ARCaP (androgen refractory prostate cancer) cells, underwent EMT, to become ARCaP$_M$ and gained increased growth and metastatic potential to bone and soft tissues (22). ARCaP$_M$ has 100% bone metastatic potential whereas ARCaP$_E$ has 12.5% (22). Accordingly, the steady-state levels of intrinsic β2-M protein were higher in ARCaP$_M$ than ARCaP$_E$ cells, as shown by western blot analysis in whole cell extracts and conditioned media (CM; Fig. 1A) and in CM by ELISA (Fig. 1B). To determine the function of β2-M we overexpressed β2-M in breast, lung, and renal human cancer cells. β2-M was overexpressed by a retroviral gene transduction method. A series of intermediate and high β2-M expressing human breast (MCF-7), lung (H358), and renal (SN12C) cancer cells were generated, characterized, and were confirmed by western blot analysis (Fig 1A) and ELISA of the CM (Fig. 1B). The high expressors of β2-M were designated MCF7/β2-M-2, H358/β2-M-2, and SN12C/β2-M-2, and the medium expressors of β2-M were designated MCF7/β2-M-1, H358/β2-M-1, and SN12C/β2-M-1 in each cell line. MCF7/P (parent), H358/P, and SN12C/P transfected with pcDNA3.1 vector alone...
MCF7/Neo, H358/Neo, and SN12C/Neo) served as controls. 

β2-M high expressors of breast, lung, and renal cancer had increased proliferation (Fig. 1C), migration, and invasion (Fig. 1D) compared to controls.

β2-M accelerated tumor growth of human breast, lung, and renal cancer with increased osteolysis in nude mice

Since ARCaPM cells were highly metastatic to bone, we compared the ability of Neo and β2-M–expressing MCF7 (breast), H358 (lung), and SN12C (renal) cancer cells to grow in the bone microenvironment in nude mice in vivo. β2-M–overexpressing clone (β2-M-2) and vector control clone (Neo) of MCF7, H358, and SN12C were injected intratibially in the mouse skeleton, and tumor growth was assessed by radiography. Figure 2A shows that larger cancer cell-induced lesions with marked osteolytic responses and spotty foci of more intense osteoblastic lesions in mouse tibias implanted with β2-M compared to Neo-expressing cancer cell clones. Tumor volumes in β2-M-2-expressing clones were on average 3.5, 4.0, and 2.7 fold bigger than the Neo-expressing clones of MCF7, H358, and SN12C, respectively (Fig. 2B).

Immunohistochemical analyses of the harvested tumors from mouse skeleton revealed increased β2-M staining in β2-M–expressing MCF7, H358, and SN12C tumors compared to Neo controls (Fig. 2C). Tartrate resistant acid phosphatase (TRAP) staining was performed to detect osteoclasts. The β2-M–expressing MCF7, H358, and SN12C cancer cells had a 3.6, 3.4, and 3.0 fold increases in osteoclasts compared to Neo controls (Fig. 2D). These results suggest that β2-M enhanced cancer cell mediated osteolysis by increasing the number of osteoclasts in breast, lung, and renal tumors grown in mouse skeleton.

β2-M expression positively correlated with the metastatic potential and lethality of human prostate, breast, lung, or renal cancer cells in immune-compromised mice

A comparative study was conducted using human prostate, breast, lung, and renal cancer cells expressing either basal or high levels of β2-M to assess cancer bone and soft tissue.
metastases and overall survival of the mice. Cells were injected intracardially into the left ventricles of nude mice. The presence of tumors in mouse skeleton and soft tissues was assessed by X-ray, physical palpation, and histopathology of tissue specimens harvested at the time of animal sacrifice. β2-M—overexpressed breast MCF7, lung H358, and renal SN12C cancer cells had significantly increased bone metastatic rates compared to controls (Table 1). β2-M—overexpressed breast MCF7, lung H358, and renal SN12C cancer cells had bone metastatic rate at 42.9% (6/14), 43.8% (7/16), and 30.8% (4/13), compared to mice inoculated with neo-expressing clones, which correspondingly were 7.1% (1/14), 6.3% (1/16), and 7.1% (1/14) (Table 1). Likewise, total soft tissue metastases to lymph nodes, liver, kidney, ovary, and adrenal glands were also moderately increased in β2-M—expressing cells of breast (MCF-7), lung (H358), and renal (SN12C) cells from 57.1%, 75%, 38.4% compared to neo-expressing controls, 35.7%, 31.2% and 21.4%, respectively (Table 1). β2-M expression was higher in metastatic bone tumors of ARCaPM and β2-M—expressing MCF7, H358, and SN12C tumors when compared ARCaPE or Neo—expressing control tumors by immunohistochemical analysis (Fig. 3A, right). Consistently, serum β2-M levels were also higher in mice injected with β2-M—overexpressing cells (Supplementary Fig. S1A). This level of β2-M is comparable to serum β2-M in human patients (27). Overall, ARCaPM and β2-M—overexpressing breast, lung, and renal tumors showed a more intense mixture of osteoblastic and osteolytic responses in bone compared with the specimens obtained from ARCaPE and Neo—expressing tumors (Supplementary Fig. S1B and C). The cumulative survival rate, as assessed by Kaplan–Meyer plots, of the mice injected intracardially with β2-M—expressing ARCaPM, MCF7, H358, and SN12C cells also had significantly poorer prognosis compared to mice inoculated with Neo—expressing cells (P = 0.0455, P < 0.0001, P = 0.0017, and P = 0.0075, respectively; Fig. 3B). These results demonstrate that β2-M overexpression alone, in cancer cells, is sufficient to drive their subsequent skeletal and soft tissue metastases and caused lethality in experimental mouse models.

### β2-M overexpression induced epithelial–mesenchymal transition in breast, lung, and renal cancer cells in vitro and in vivo

Both clinical and experimental data support the notion that cancer cells gain their metastatic potential by undergoing EMT (28). Using a robust ARCaP EMT model, we demonstrated a close association between EMT and prostate cancer bone metastasis (Fig. 4A). As a consequence of β2-M overexpression in breast, lung, and renal cancer cells, we observed notable EMT morphologic changes (Fig. 4A). β2-M—expressing ARCaPM, MCF7, H358, and SN12C had decreased E-cadherin and increased N-cadherin and vimentin, compared to their neo—expressing control tumors (Fig. 4D). Similar results were observed in intratibial tumor tissue sections harvested from mice inoculated with the β2-M—expressing and neo—expressing cell clones (Supplementary Fig. S2). These results support the concept that EMT occurred subsequent to β2-M expression and this phenotype is stable in vivo.
β2-M interacts with hemochromatosis (HFE) protein and inhibition of β2-M or HFE reverts EMT

To determine if inhibition of β2-M could reverse EMT [i.e., induce mesenchymal to epithelial transition (MET)], we performed studies knocking down intracellular β2-M with β2-M sequence-specific siRNA in ARCaPM prostate cancer cells. The control cells were treated similarly, using scrambled siRNA sequence (Scram). β2-M knockdown cells (KDI and KDII) had lower β2-M protein (Fig. 5A) and mRNA (Supplementary Fig. S3A) compared to ARCaPM Scram control. Both KDI and KDII underwent stable morphologic mesenchymal to epithelial transition (MET; Fig. 5B), which was accompanied by increased E-cadherin and decreased vimentin expression (Fig. 5A). Decreased β2-M also resulted in decreased invasion and migration (Supplementary Fig. S3B). HFE has been previously known to interact with β2-M. We tested in β2-M and HFE complex exists in prostate cancer cells. Physical interaction between β2-M and HFE as a complex was demonstrated by coimmunoprecipitation (co-IP) followed by western blot analyses (Fig. 5C). To determine the possible functional roles of β2-M/HFE complex-mediated EMT in ARCaPM cells, we knocked down HFE protein using HFE shRNA lentiviral constructs. Several stable clones were generated and KD_HFE1 and KD_HFE3 knockdown were used for further EMT characterization. KD_HFE1 and KD_HFE3 had significantly decreased HFE protein levels (Fig. 5D). Decreased HFE protein also resulted in decreased expression of vimentin and a moderately increased expression of E-cadherin (Fig. 5D, Supplementary Fig. S4D). Decrease in HFE also downregulated the expression levels of β2-M, thus reducing the β2-M/HFE complexes. Inhibition of HFE in C4-2 prostate cancer cells using a similar method resulted in decreased HFE and in increased E-cadherin (Supplementary Fig. S4A). We observed that downregulating HFE protein switched the morphology of ARCaPM cells to a cobblestone-like appearance, much like ARCaPE cells (Fig. 5D). Thus, disrupting the function of the β2-M/HFE complex by either HFE or β2-M knockdown is sufficient to reverse β2-M mediated EMT in prostate cancer cells. HFE-knockdown ARCaPM and C4-2 cells also had decreased invasive and migratory activity compared to control cells (Supplementary Fig. S4B and C).

Iron modulated EMT in cancer cells

β2-M protein is known to directly regulate iron levels in cells, in which β2-M/HFE complex block transferrin receptor 1 and prevent iron uptake. β2-M and HFE knockout mice have
We hypothesized that b2-M overexpression in ARCaPM cells decreases iron and induces iron responsive HIF-1α (29). HIF-1α was previously shown to be elevated in mesenchymal ARCaPM cells compared to epithelial ARCaPE cells under normoxic conditions (30). We tested if cellular iron levels were lower in b2-M higher-expressing ARCaPM cells compared to b2-M lower-expressing ARCaPE cells and in HFE knockdown cells, using inductively coupled plasma mass spectroscopy (ICP-MS). Intracellular iron was significantly lower in ARCaPM compared to ARCaPE cells, KDHFE1 and KDHFE3 knockdown cells (Fig. 6A). To determine if iron could regulate EMT we used iron chelator to induce EMT like changes. Since the epithelial cancer cells (ARCaPE, KDHFE1, and KDHFE3 knockdown cells) had slightly higher basal iron compared to ARCaPM, we used iron chelator (deseral) on ARCaPE cells. Iron chelation increased HIF-1α and induced mesenchymal characteristics [Fig 6B(i) and (ii)]. We tested if HIF-1α can promote EMT in ARCaPE cells in response to hypoxic conditions. Hypoxia, upregulated HIF-1α, and ARCaPE cells exhibited mesenchymal like characteristics compared to cells maintained under normoxic conditions [Fig. 6C(i) and –(ii)]. b2-M knockdown cells had decreased HIF-1α measured by intracellular flow cytometry (Supplementary Fig. S3C). These results collectively demonstrate that b2-M expression in ARCaPM cells leads to decreased iron and increased HIF-1α, which induces EMT in prostate cancer cells.

In summary, b2-M can drive EMT, increase cancer bone and soft tissue metastasis and cause death in mice. b2-M mediates this process by interacting with a b2-M receptor, HFE, which together control intracellular iron homeostasis, activating HIF-1α, to promote EMT and increase lethal cancer cell metastases (Fig. 6D).

Discussion

The role of b2-M has long been documented in several solid and liquid cancers, but its mechanism of action is poorly understood. In this study, we documented for the first time that b2-M overexpression can drive EMT and promote the growth, invasion, and metastasis of human prostate, breast,
lung, and renal cancer cells in vitro and in vivo and cause lethality in mice. We showed that (i) β2-M promoted EMT and its associated increase in cancer cell proliferation, migration, and invasion in vitro, and caused lethal skeletal and soft tissue metastases in mice; (ii) β2-M induced stable expression of EMT biomarkers, including decreased expression of E-cadherin and increased expression of N-cadherin and vimentin in cancer cells grown as primary and metastatic tumors in experimental mouse models; and (iii) β2-M forms a complex with its receptor HFE, which regulates intracellular iron and activates HIF-1α in cancer cells. To our knowledge, this is the first report to demonstrate how β2-M functionally confers increased cancer bone and soft tissue metastases in human prostate, breast, lung, and renal cancer cells by its induction of EMT in these cancer cells.

β2-M is a known growth-promoting protein for prostate (7, 10) and multiple myeloma (11) cells as well as normal bone cells, osteoblasts (17), osteoclasts (18), prostate stromal cells (10), and mesenchymal stem cells (16). β2-M was shown to promote osteomimicry in prostate cancer cells, allowing them to grow and survive in hostile bone microenvironments (7). Therefore it is not surprising that β2-M-overexpressing clones of prostate, breast, lung, and renal cancers had significantly increased bone metastases (Table 1) and lethality in experimental animals (Fig. 3B). β2-M may favor bone metastasis because firstly, increased β2-M expression in cancer cells promotes increased expression of bone matrix proteins such as osteocalcin and bone sialoprotein, mimicking the bone “niche” and supporting the growth and survival of prostate cancer cells in the bone microenvironment (7). Secondly, increased serum β2-M has been associated with increased bone remodeling which could trigger the secretion of soluble and matrix factors feeding further growth of cancer cells in the skeleton. Thirdly, β2-M could also promote the growth of osteoclasts (Fig. 2D), osteoblasts (31), and migrating mesenchymal stem cells (16) in the tumor microenvironment, further enhancing the growth of primary and metastatic cancer cells (32). Fourthly, β2-M could contribute to iron homeostasis and induction of HIF-1α in cancer cells (Fig. 6B and C) to promote the growth of cancer in the skeleton. Finally, β2-M has been proposed as a coupling factor between osteoclasts and osteoblasts (33) with a role in augmenting tumor and marrow stroma interaction, which could further activate a vicious cycle of metastatic cancer progression in bone (34).

β2-M mediates several hallmarks of malignancy, such as self-renewal capabilities, by activating phosphorylated cAMP response element binding protein, cyclin D1, and cyclin A (7), evading apoptosis by recruiting survival and growth factors and their receptors for downstream signaling (35), enhancing angiogenesis by activating VEGF-neuropilin signaling (7, 36), and inducing resistance to treatment and increasing stemness by activation of the HIF-1α signaling pathway (37). HIF-1α overexpression in tumor specimens is correlated with patient mortality (38). β2-M is upstream of HIF-1α, and induces a hypoxia-like effect through the reduction of iron levels. Here, we demonstrated that β2-M induces EMT and stemness-like properties in cancer cells.
In contrast to multiple myeloma, which expresses normal levels of MHC class 1 family members, β2-M interacts with MHC class 1 and mediates its downstream signaling processes by sequestering growth and survival signaling components mediated by lipid membrane and lipid rafts (11). In solid tumors, however, MHC class 1a members involved in antigen presentation are frequently downregulated. Thus MHC class 1b members, known to be involved in nonimmunological activities, are likely to mediate the β2-M downstream signaling functions of these tumor cells. HFE, a MHC class 1b protein shown to have a smaller groove and unable to present antigens (39), is likely to assume the signaling role of β2-M. β2-M/HFE has been shown to regulate negatively intracellular iron, activate HIF-1α and drive EMT in cancer cells. Our studies demonstrated that HFE is a β2-M receptor, since: (1) HFE was found to physically interact with β2-M, demonstrated by immunoprecipitation in prostate cancer cells (Fig. 5C) and (2) knocking down either HFE or β2-M resulted in MET, a reversal of EMT, in prostate cancer cells with supportive morphologic, biochemical, and behavioral characteristics. Thus β2-M/HFE interactions are important for β2-M mediated EMT and cell survival. The downstream functional significance of the β2-M/HFE complex is depicted in Figure 6D. β2-M/HFE plays a key role in regulating iron homeostasis in cancer cells, mediated by interacting with TFRC (transferrin receptor complex 1). β2-M protected the influx and accumulation of intracellular iron. Higher β2-M/HFE levels downregulated intracellular iron levels in ARCaP M cells and low levels of β2-M/HFE complex in ARCaPE cells enhanced intracellular iron levels (Fig. 6A). Lower levels of intracellular iron activated HIF-1α and its target genes in ARCaPM cells, driving EMT (30), which could contribute to resistance to treatments such as radiation and chemotherapy, resistance to apoptosis and increased angiogenesis (40). HIF-1α modulates the cell’s redox balance by generating large levels of redox buffers such as glutathione and thioredoxin and alternatively activating NADPH oxidase enzymes as ROS generator and signaling molecules (37).

In summary, we demonstrated the importance of β2-M for cancer cell growth, invasion and metastasis. The action of β2-M is mediated by forming a complex with HFE which regulates intracellular iron homeostasis and HIF-1α and ultimately cancer metastasis to bone and soft tissues. The cell signaling network mediated by β2-M/HFE complex is highly conserved among several cancer cell types and deregulation of this complex is depicted in Figure 6D. β2-M/HFE modulate iron levels to induce EMT. A, iron levels in ARCaP M and ARCaP A cells and HFE knockdown cells (KDHFE1 and KDHFE3) measured by inductively coupled plasma mass spectroscopy. B, EMT changes in ARCaP M cells in response to iron chelation: (i) morphological changes and (ii) expression changes in EMT biomarkers. C, EMT changes in ARCaP M cells in response to hypoxia: (i) morphological changes and (ii) expression changes in EMT biomarkers. D, model of β2-M mediated bone metastasis. The β2-M/HFE complex modulated iron uptake by negative regulation of transferrin receptor 1. The β2-M/HFE complex maintains low iron levels and activates the EMT program via iron responsive pathways such as HIF-1α signaling. β2-M induces EMT and allows increased bone metastasis in prostate, breast, lung, and renal cancers.
complex could affect cancer growth and lethality in mice by the induction of EMT.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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