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CABS OP1.1 (P133)

Cripto/GRP78 Signalling in Dissemination and Metastasis of Human Osteotropic Prostate Cancer

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Prostate cancer is the most prevalent cancer in men and metastatic spread to bone is detected in up to 80% of the patients with advanced disease. Cell surface oncoproteins are attractive therapeutic targets, readily accessible to antibodies and other membrane impermeable protein/peptide-based anti-cancer agents. Cripto is a GPI-anchored cell surface/secreted oncoprotein that plays important roles in embryogenesis, stem cell maintenance and tumour progression. GRP78 is a HSP70 family member that binds Cripto at the cell surface. We recently found that Cripto and GRP78 are both highly expressed in human castration resistant prostate cancer (PCa), but not in androgen-dependent tumours. We investigated if Cripto/GRP78 signalling promotes the aggressive, stem cell-like PCa phenotype associated with castration resistance and bone metastasis. To mimic the endosteal metastatic niche, highly metastatic human PC-3M-Pro4luc2 prostate cancer cells were cultured with primary human osteoblasts. We found that the presence of human osteoblasts reduces the proliferation of PC-3M-Pro4luc2 cells and results in induction of the E-Cadherin repressor ZEB1, causing the PCa cells to acquire a more mesenchymal, invasive phenotype as reflected by their reduced E-Cadherin/Vimentin ratio. Co-culture of PC-3M-Pro4luc2 cells with osteoblasts also greatly increased the ALDH^{high}/ALDH^{low} ratio indicating an increase in the size of the metastatic stem/progenitor cell population. This increase in the ALDH^{high} subpopulation corresponded to enhanced Cripto and GRP78 expression and stable knockdown of Cripto or GRP78 reduced PC-3M-Pro4luc2 proliferation and clonogenicity, and decreased the size of the metastasis-initiating ALDH^{high} subpopulation. Finally, we used zebrafish as a model system for measuring tumour cell dissemination and metastasis and found that Cripto knockdown in PC-3M-Pro4luc2 cells led to a significant reduction in metastatic tumour burden. In conclusion, our findings point to a potential role for Cripto and GRP78 in driving metastatic, therapy-resistant phenotype and suggest that targeting the Cripto/GRP78 pathway may have significant therapeutic potential.

CABS OP1.2 (P132)

Contribution of Osteocytes to Cancer-Associated Bone Pain via Connexin43-Mediated Communications with Sensory Neurons Under the Acidic Microenvironment in Bone Metastasis

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Bone pain is one of the most prevalent and devastating complications of cancer in bone. The pathophysiology of cancer-associated bone pain (CABP) is poorly understood but likely involves complex interactions among the cancer cells, peripheral sensory nerves and bone cells. Recent studies reported that the calcitonin gene-related peptide-positive (CGRP+) sensory neurons densely innervate mineralised bone, in which numerous osteocytes are present, leading us to hypothesise that osteocytes interact with these CGRP+ sensory neurons to evoke CABP. We tested this hypothesis using an animal model in which inoculation of the JLN3 human multiple myeloma (MM) cells into tibiae induced progressive CABP. We found that JLN3 MM-colonised bone was acidic and that blockade of the acidification by the proton pump inhibitor bafilomycin A1 significantly reduced the CABP. Immunohistochemical examination demonstrated that osteocytes localised in the close proximity of CGRP+ primary afferent sensory neurons in mineralised bone. Co-culture of MLO-A5 osteocytic cells and F11 sensory neuronal cells showed that MLO-A5 cells transferred the permeable living dye calcein to F11 cells by extending dendritic processes to contact the neurites of F11 cells. The general gap junction inhibitor 18β-GA and the selective connexin43 (Cx43) blocker GAP27 and silencing Cx43 in MLO-A5 cells by shRNA all decreased the dye transfer, suggesting that the Cx43 gap junction mediates the osteocyte-sensory neuron communication. Determination of neuronal excitation by Ca²⁺ influx imaging assay showed that the acidic medium excited F11 sensory neuronal cells. Importantly, acid-induced excitation of F11 cells was enhanced in the presence of MLO-A5 osteocytic cells and GAP27 and silencing Cx43 abolished acid-induced F11 excitation in the co-cultures. In conclusion, our results suggest that osteocytes contribute to the pathophysiology of CABP via Cx43-mediated communications with sensory neurons innervating bone. These communications may be a novel therapeutic target in the management of CABP.

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CABS OP1.3 (P136)**Endothelin-1, a Gene Regulated by TMPRSS2:ERG Fusion Proteins in Prostate Cancer Bone Metastases**

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Bone metastases are frequent and severe complications of prostate cancer (PCa). Recently, the *TMPRSS2:ERG* gene fusion, which results in the aberrant androgen-dependent expression of the ERG transcription factor, has been shown to be the most common gene rearrangement in PCa. This study investigates a potential role of the gene fusion in the development and phenotype of PCa bone metastases. We previously established cell clones from a PCa cell line (PC3c), over-expressing different levels of *TMPRSS2:ERG*. *In vivo* analysis of bone lesions induced by intra-tibial injections of PC3c-*TMPRSS2:ERG* clones in mice (ethical approval DR2014-32) showed an increase of osteoblastic phenotype compared with control cells. Furthermore, a transcriptomic study of these clones showed a change of expression in many genes, including *endothelin-1* (*ET-1*). Since *ET-1* is known to be involved in osteoblast proliferation and in osteoblastic metastasis formation in PCa, we therefore investigated the transcriptional regulation of *ET-1* by fusion proteins. *In vitro*, we have shown that this gene was overexpressed in PC3c-*TMPRSS2:ERG* clones, depending on ERG expression levels, and was inhibited by ERG silencing. *In silico* analysis of the promoter of *ET-1* revealed the presence of several potential binding sites of ERG. Chromatin immunoprecipitation experiments demonstrated a direct binding to one of them. Moreover, using a cohort of human carcinoma prostate samples (ethical approval CSTMT-042), we were able to establish a correlation between the expression of *ET-1* and the expression of the fusion gene *TMPRSS2:ERG*, reinforcing the link between *ET-1* and the fusion. Taken together, these results strongly suggest that the *TMPRSS2:ERG* gene fusion contributes to the osteoblastic phenotype of PCa bone metastases and that *ET-1* is a crucial target gene regulated by the transcription factor ERG.

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CABS OP3.1 (P131)**The Inhibition of c-MET Reduces Bone Metastases Induced by Renal Cancer Stem Cells**

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Renal cancer patients often develop particularly destructive bone metastases. In solid tumours, cancer stem cells (CSCs) directly promote bone metastasis, thus therapeutic strategies to block the interaction between CSCs and bone micro-environment are currently under investigation. Since c-MET mediates the interaction between cancer cells and mesenchymal cells of the bone microenvironment, we hypothesised that targeting c-MET will lead to bone metastases inhibition. Renal CD105+ CSCs isolated from human cancer patients were injected in NOD/SCID mice, previously implanted with a small fragment of human bone. After the injection of CSCs, mice were daily treated or not with a c-MET inhibitor (JNJ) for 90 days, then sacrificed. Importantly renal CSCs colonised human implanted bone but not mice bone, leading to a specie-specificity of those cells to metastasize human bone. We then found that the JNJ treatment inhibited metastatisation at bone implant site. We studied the effect of JNJ on osteoclasts (OCs) and osteoblasts (OBs) of the bone implant by histomorphometry, showing that CSCs induced an activation of OCs corresponding to an increase erosion surface, whereas the OB activity diminished with a reduction of the osteoid thickness. The treatment with JNJ restored the normal activity of OCs and OBs, comparable with the control mice. Then we investigated the effect of JNJ on *in vitro* cultures of human OCs and OBs, to avoid the bone micro-environment interference. JNJ reduced the number of TRAP+ OCs, whereas it did not significantly affect the number of BAP+ OBs. Furthermore, we analysed mice sera by a multi-analyte detection system, showing that IL-11 and CCL20 levels are higher in mice untreated with JNJ than in treated ones, suggesting a role of these molecules in the CSC bone metastatic process. Our results highlight the ability of this c-MET inhibitor to abrogate the bone metastasis formation induced by renal CSCs.

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CABS OP3.2 (P134)**Calpain-6 Expression Identifies a Stem Cell Population in Osteosarcoma**

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Identification of cancer stem cells in carcinomas has proved to be useful to understand cancer progression and for prognostic purpose. However, the properties of osteosarcoma stem cells remain challenging and controversial, mainly due to the lack of functional markers to study such cells *in vivo*. Previously, we identified calpain-6 as a protective factor involved in chemoresistance process of osteosarcoma. To investigate the mechanisms controlling its expression we characterised 7285 bases of the regulatory sequence in calpain-6 gene. This sequence comprises an active promoter and multiple functional binding sites for embryonic stem cell factors such as Oct4, Nanog and Sox2 as shown by rapid cDNA end amplification and chromatin precipitation. Silencing Oct4, Nanog or Sox2 was sufficient to reduce basal and hypoxia dependent up-regulation of calpain-6 expression and the activity of the regulatory sequence cloned upstream the luciferase gene reporter. This indicates that calpain-6 is controlled by the stem cell transcription factors. To further document a possible relationship between Calpain-6 and a stem cell phenotype, we used GFP as gene reporter, to identify the cells in which the calpain-6 promoter was activated. Culturing osteosarcoma cell lines on non-adherent plastic and in minimal medium allowed obtaining spheroids that were previously shown to be enriched in tumorigenic stem-like cells. Calpain-6 protein was up regulated in spheres obtained from human 143B cells as compared with adherent cultures. Moreover, GFP positive cells sorted from adherent cultures have higher capacities to form spheroids than GFP negative cells. These GFP positive cells also expressed higher RNA levels of the embryonic stem cell markers, c-MYC and ABCB1. Five weeks after injection into the tibia of BALB/c mice, GFP-positive K7M2 cells formed tumours that produced a high luminescent signal as compared with tumours formed from GFP-negative cells that are largely necrotic. In *in vitro* scratch tests, migrating cells were found to express high levels of calpain-6 and GFP-positive cells displayed higher capacities for migration than negative ones, whereas, calpain-6 shRNA reduced these capacities. Finally, intra bone injection of GFP-positive cells resulted in more metastatic lesions in lungs than negative cells indicating that calpain-6 is involved in metastatic process. Altogether, our data show that calpain-6 expression is regulated by transcription factors that control multipotency and renewal of embryonic stem cells. Calpain-6 identifies an osteosarcoma cell population that express stem markers and with higher chemoresistance, migration capacities and tumorigenicity. The reporter system driven by calpain-6 regulatory sequence may therefore represent a powerful tool to further study stem cells in osteosarcoma.

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CABS OP3.3 (P137)**EPCR Promotes a Tumorigenic and Metastatic Phenotype in Breast Cancer**

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Endothelial protein C receptor (EPCR) is a transmembrane receptor widely expressed in endothelial cells where it exerts cytoprotective and anticoagulant activities. We have shown that it is also expressed in lung tumour cells where it promotes tumour cell survival and increases osseous prometastatic activity. However, to date the contribution of EPCR to tumourigenesis and skeletal metastasis in breast cancer remains ill defined. Lentiviral shRNA-mediated EPCR silenced (shEPCR) cells in the MDA-MB-231 derived 1833 breast cancer cell line showed unaltered growth kinetics in basal or apoptotic-induced *in vitro* conditions. However, EPCR silencing reduced tumour growth in an orthotopic model of mammary fat pad injection. Interestingly, intracardiac inoculation of shEPCR cells led to a substantial reduction in skeletal metastatic burden, assessed by bioluminescence imaging, and osteolytic lesions, evaluated by micro-X-Ray imaging, micro-CT scans and histological analysis. This effect was associated with a decreased skeletal tumour growth observed after intratibial inoculation of shEPCR cells as compared with control cells. Furthermore, after intra-tail injection of the murine breast cancer cell line ANV5, we found a dramatic decrease in lung metastasis in animals injected with shEPCR cells as compared with control mice, despite the similar growth kinetics of the cell lines *in vitro*. *In vivo* transcriptomic analysis identified several relevant signalling pathways differentially altered in shEPCR and control tumours. To explore the clinical relevance of these findings we carried out global expression analysis in a cohort of 286 patients. Patients with high EPCR expression levels had shorter relapse-free survival times as compared with patients with low EPCR expression levels. These data indicate that EPCR confers an *in vivo* protumorigenic and prometastatic phenotype to bone and lung. Monitoring EPCR could represent a clinically relevant factor in breast cancer and a potential therapeutic target.

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CABS OP3.4 (P130)**Bone Cells Control Myeloma Cell Dormancy and Activation in the Skeleton**

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Multiple myeloma predominantly grows in bone, causing extensive destruction. Despite targeted therapies, relapse is common and the disease remains incurable. To develop more effective treatments we need an improved understanding of

myeloma cell engraftment, dormancy and reactivation in the skeleton. We hypothesise that myeloma cells engage in an endosteal niche in which they reside in a dormant state, resist chemotherapy and can be reactivated through changes in the local environment, contributing to disease relapse. To address this, we have developed *intravital* imaging to study tumor cell colonization of the endosteal niche and tumour cell dormancy and reactivation. 5TGM1eGFP murine myeloma cells were labelled with the membrane dye DiD. *In vitro*, DiD label is lost through division, distinguishing dormant (DiD⁺/GFP⁺) from proliferating cells (DiD⁺/GFP⁺). Myeloma cells were injected into C57BLKwRij mice and treated with melphalan (3 times/week, 5mg/kg days 14-28), sRANKL (daily days 4-6) or vehicle. Using *intravital* microscopy, individual dormant DiD⁺/GFP⁺ cells were visualised at 7, 14, 21, or 28 days post injection and located in endosteal niches. By day 14, a limited number of myeloma cells were activated to form growing DiD⁺/GFP⁺ colonies which were localised distant from bone surfaces. Melphalan treatment reduced tumour burden (<97%), however dormant DiD⁺/GFP⁺ tumour cells remained. Following removal of melphalan treatment, tumor burden increased and DiD⁺/GFP⁺ cells reduced, indicating that re-activation had occurred. Lastly, sRANKL stimulation of osteoclast activity reduced dormant (DiD⁺/GFP⁺) cells, suggesting osteoclast driven increased reactivation of dormant tumour cells. Taken together, these data show that dormant tumour cells, which reside in endosteal niches, resist chemotherapy and are available to repopulate the tumour. Importantly, we demonstrate that increased osteoclast remodelling of the endosteal niche reactivates tumour cells in the skeleton. These data provide insights into the fate of dormant cells, mechanisms behind drug resistance and identifies new mechanisms for disease relapse.

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CABS OP3.5 (P138)

Hif Signalling in Skeletal Progenitors Promotes Breast Cancer Growth and Metastasis Through Systemic Production of CXCL12

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High bone mineral density (BMD) has long been associated with increased risk of breast cancer. Conversely, low bone mass has been correlated with lower risk of breast cancer. Although BMD was initially thought to reflect a cumulative exposure to oestrogens, recent clinical trials demonstrated that high bone mass correlates with elevated breast cancer incidence independently of reproductive correlates, endogenous and exogenous exposure to oestrogen. However, the biological mechanism linking bone mass and the risk of breast cancer is unknown. Our objective was to investigate the role of the osteoblastic lineage in breast cancer, using transgenic mice presenting increased or decreased bone mass (all animal protocols were approved by an animal ethics committee). Here we show that osteoprogenitor cells, targeted by Osterix driven Cre-recombinase, exert a systemic control of breast cancer

growth and metastasis. Deletion of the tumour suppressor gene von Hippel Lindau (Vhlh) specifically in mouse osteoprogenitors (Osx/Vhlh^{fl/fl}), which results in increased protein level of the Hypoxia-Inducible Factor-1alpha (Hif-1alpha) in these cells, led to increased bone mass, and increased mammary tumour growth and metastasis. Conversely, deletion of Hif-1alpha in osteoprogenitors (Osx/Hif-1alpha^{fl/fl}) decreased bone mass, and dampened mammary tumour growth and metastasis. We found that changes in the bone microenvironment are associated with changes in the plasmatic levels of the chemokine C-X-C motif ligand 12 (CXCL12). Pharmacological inhibition of the CXCL12-CXCR4 pathway abolished increased primary tumour growth and dissemination in Osx/Vhlh^{fl/fl} mice. Therefore, skeletal dysfunction alters tumorigenesis beyond the bone microenvironment. Our results provide a mechanistic explanation as for why high bone mass is linked to increased risk of breast cancer, and support the notion that the skeleton is an important organ of the tumour macroenvironment. They also indicate that drugs affecting bone homeostasis may have important consequences in breast cancer.

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CABS OP4.1 (P129)

miR-25 and miR-21 Regulate Prostate Cancer Invasiveness by Attenuation of Notch-TGF-β Crosstalk and Self-Renewal Markers

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Altered microRNA (miR) expression is associated with tumour formation and progression of various solid cancers. A major challenge in miR profiling of bulk tumours is represented by the heterogeneity of the subpopulations of cells that constitute the organ and tumour tissue. We analysed the expression of miRs in a subpopulation of bone metastasis-initiating stem/progenitor-like cells in human prostate cancer (PCSC) and compared with more differentiated cancer cells. In PC-3M-Pro4Luc2 and C4-2B prostate cancer cell lines and clinical prostate cancer specimens we identified that miR-25 and miR-21 expression in PCSCs was low/absent and steadily increased during their differentiation into cells with a luminal epithelial phenotype. Functional studies revealed that overexpression of miR-25 in prostate cancer cell lines and selected subpopulation of highly metastatic/tumorigenic cells (ALDH^{high}) strongly affected the invasive cytoskeleton reducing migration *in vitro*, while overexpression of miR-21 reduced the size of ALDH^{high} subpopulation. Additionally, miR-25 overexpression dramatically decreased the expression of Notch1 and Jagged1, critically involved in aetiology of skeletal metastasis, together with other Notch downstream targets in prostate cancer cells, while miR-21 downregulated self-renewal markers.

Moreover, we found that miR-25 decreased TGF- β signaling in human prostate cancer cells and that miR-25 overexpression blocks the induction of Jagged1 driven by TGF- β . In line with these observations, we further demonstrate that miR-25 can act as a tumour suppressor in highly metastatic PCSCs by direct functional interaction with the 3'UTR of pro-invasive α_6 and α_v - integrins. Finally, we show here for the first time, that miR-25 can reduce metastasis by blocking the extravasation of human prostate cancer cells *in vivo*. Taken together, our observations suggest that miR-21 and miR-25 are key regulators of invasiveness in human prostate cancer through direct interactions with α_v - and α_6 integrins & Notch1 expression.

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CABS OP4.2 (P128)

Targeting Runx2 By Mir-135 and Mir-203 Impairs Breast Cancer Metastasis and Progression of Osteolytic Bone Disease

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Progression of breast cancer to metastatic bone disease is associated with an aberrantly elevated expression of Runx2, which promotes disease progression through transcriptional activation of genes involved in metastasis. Inhibition of Runx2 in metastatic breast cancer cells prevents metastatic bone disease, thus providing a basis for Runx2 as a potential therapeutic target. Since transcription factors are challenging to target for therapeutic intervention, our goal was to evaluate the potential clinical use of Runx2-targeting microRNAs (miRNAs) to reduce tumour growth and bone metastatic burden. Expression analysis of a panel of miRNAs regulating Runx2 revealed a reciprocal relationship between the abundance of Runx2 protein and two miRNAs, miR-135 and miR-203. These miRNAs are highly expressed in normal breast epithelial cells where Runx2 is not detected, and conversely are absent in metastatic breast cancer cell lines and importantly, in tissue biopsies that express Runx2. Reconstituting metastatic MDA-MB-231-luc cells with miR-135 and miR-203 reduced the abundance of Runx2 and the expression of the metastasis-promoting Runx2 target genes. Additionally, tumour cell viability was decreased and migration suppressed *in vitro*. *In vivo* implantation of MDA-MB-231-luc cells reconstituted with miR-135 or miR-203 into the mammary gland, followed by additional intratumoural administration of the synthetic miRNAs reduced tumour growth and importantly, spontaneous metastasis to bone. Furthermore, intratibial injection of

these cells impaired tumour growth in the bone environment, inhibited bone resorption and secondary metastasis to lung. Importantly, reconstitution of Runx2 in MDA-MB-231-luc cells delivered with miR-135 and miR-203 reversed the inhibitory effect of the miRNAs on tumour growth and metastasis. We conclude that aberrant expression of Runx2 in aggressive tumour cells is related to the loss of specific Runx2-targeting miRNAs and that a clinically relevant replacement strategy by delivery of synthetic miRNAs is a viable therapeutic approach to target transcription factors for the prevention of metastatic bone disease.

Disclosure: The authors declared no competing interests.

CABS OP4.3 (P139)

Radium-223 Dichloride Exhibits Dual Mode-of-Action Inhibiting both Tumour and Tumour-Induced Bone Growth in Two Osteoblastic Prostate Cancer Models

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Radium-223 dichloride, an alpha particle-emitting calcium-mimetic, improves overall survival in prostate cancer patients with symptomatic bone metastases. Here, we define radium-223 mode-of-action and efficacy in two clinically relevant prostate cancer xenograft models. Human LNCaP or patient-derived LuCaP 58 prostate cancer cells were inoculated intratibially and mice were stratified into treatment groups based on lesion grade and/or serum PSA levels. Radium-223 (300 kBq/kg) or vehicle was administered twice at 4-week intervals. X-rays and serum samples were obtained biweekly. Bone samples were collected for γ -counter measurements, micro-CT, autoradiography and histology. Radium-223 inhibited tumour-induced osteoblastic reaction as indicated by reduced bone volume and surface area in both prostate cancer models. Additionally, radium-223 suppressed metabolic activity in bone as evidenced by decreased osteoblast and osteoclast numbers and reduced PINP levels. Radium-223 treatment also resulted in lower PSA levels as early as two weeks post first dosing, indicating constrained tumour growth. This phenomenon was further supported by reduced tumour area in tibia in both models and an overall increase in necrotic tumour area in the LuCaP 58 model. Moreover, DNA double-strand breaks were increased in cancer cells 24 hours post radium-223 administration in the LuCaP 58 model providing further evidence of anti-tumour effects. Autoradiography confirmed radium-223 deposition in the intratumoural bone matrix in conjunction with osteoblasts. We demonstrate that radium-223 dichloride is successfully incorporated into the intratumoural bone matrix and inhibits tumour growth in both cell line- and patient-derived osteoblastic prostate cancer models. Importantly, given the α -particle range of 50-80 μ m, potent radiation effects on the immediate tumour microenvironment are expected with minimal or no effects on the more distant bone marrow. Taken together, radium-223 therapy exhibits

a dual mode-of-action that impacts on tumour growth and tumour-induced bone reaction, both important players in the destructive vicious cycle of osteoblastic bone metastasis in prostate cancer.

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CABS OP4.4 (P135)

Salinomycin Treatment Inhibits Prostate Cancer Growth *In Vitro*, *In Vivo* and in Near-Patient *Ex Vivo* Models

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Prostate cancer (PCa) is the most common cancer in men, and up to 70-80% of patients with advanced disease present with bone metastases. Current treatment options for metastasised PCa are not curative since hormone, chemo-, and radiation-therapy are relatively ineffective in targeting PCa cells with stem/progenitor-like characteristics (CSCs). Salinomycin, an antibiotic used in poultry, was previously identified in a high through-put screen to target breast CSCs 100x more effec-

tively than paclitaxel. In this study we investigated the anti-tumour effects of salinomycin in human PCa cells *in vitro*, *in vivo* and *ex vivo*. Salinomycin dose-dependently inhibited the proliferation of various human PCa cells (PC3, PC-3M-Pro4, DU145, C4-2B, PC339, PC346C). Interestingly, after establishing docetaxel-resistant cells by serial passaging *in vivo* (PC339-DOC), salinomycin differentially affected docetaxel-resistant cells (vs. parental). Salinomycin induced apoptosis as determined by flow cytometry (Ann/PI) and immunohistochemistry (caspase-3), reduced Notch-signalling (RBPkJ/Luc reporter assay) and inhibited migration of PC-3M-Pro4 cells. When PC-3M-Pro4 cells were FACS-sorted for high aldehyde dehydrogenase (ALDH) enzymatic activity, salinomycin inhibited the clonogenic and sphere-forming capacity of both CSC and non-CSCs equally well. Salinomycin pretreatment of PC-3M-Pro4/mCherry completely blocked extravasation and metastatic colonisation in a zebrafish model with a GFP+ vasculature in which cells were intravascularly injected. Salinomycin pretreatment of PC-3M-Pro4/luc2 cells also reduced the formation of distant metastases in a bone metastasis model of intracardiac injection of cancer cells in nude mice. *Ex vivo*, salinomycin treatment for 7 days (vs. vehicle treated) strongly reduced the number of PCa cells in a novel 'near-patient' model of culturing prostate tumor slices from transurethral resection of prostate cancer tissue (TURP) and bone metastases. In conclusion, salinomycin is effective in inhibiting PCa growth *in vitro*, *in vivo* and in near-patients *ex vivo* models. Therefore, salinomycin may be a promising novel therapeutic approach for the treatment of advanced, bone metastatic PCa.

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