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CABS PARALLEL PROGRAMME

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CABS 1.1

Coupling of Angiogenesis and Osteogenesis by a Specific Vessel Subtype in Bone

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The mammalian skeletal system harbours a hierarchical system of mesenchymal stem cells, osteoprogenitors and osteoblasts sustaining lifelong bone formation. Osteogenesis is indispensable for the homeostatic renewal of bone as well as regenerative fracture healing, but these processes frequently decline in ageing organisms leading to loss of bone mass and increased fracture incidence. There is evidence indicating that the growth of blood vessels in bone and osteogenesis are coupled, but relatively little is known about the underlying cellular and molecular mechanisms. Here we identify a new capillary subtype in the murine skeletal system with distinct morphological, molecular and functional properties. These vessels are found in specific locations, mediate growth of the bone vasculature, generate distinct metabolic and molecular microenvironments, maintain perivascular osteoprogenitors, and couple angiogenesis to osteogenesis. The abundance of these vessels and associated osteoprogenitors was strongly reduced in bone from aged animals, which was pharmacologically reversible to restore bone mass.

CABS 1.2

Molecular Stromal Signatures in the Supportive Bone Microenvironment of Breast and Prostate Cancer

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Cancer cell growth is highly dependent on a growth permissive microenvironment (stroma). Prostate and mammary cancer (PCa and MCa) cells preferentially metastasize to bone, where they induce either an osteoblastic or osteolytic response. These opposite stromal responses suggest that different cancers adopt distinct strategies to hijack the bone marrow/bone stroma for their growth support. However, the molecular cues underlying these divergent responses are largely elusive. We exploited the sufficient divergence between human and mouse RNA sequences to dissect the stroma (mouse) from the cancer cell (human) transcriptome in bone metastasis xenograft models of human osteoinductive PCa cells (VCaP and C4-2B) and of pro-osteolytic PCa and MCa cells (PC-3 and MDA-MB-231, respectively). A robust induction of genes involved in osteogenesis and angiogenesis dominates the stroma response in osteoblastic bone metastasis. This translates in an amplification of hematopoietic and, remarkably, prostate epithelial stem cell niche components that may function as a self-reinforcing bone metastatic niche. The induction of this combinatorial stem cell niche is a novel mechanism that may also explain cancer cell osteotropism and the local interference with hematopoiesis (myelophthisis). Angiogenesis and skeletogenesis are the predominant biological processes also in the stroma response to osteolytic bone metastasis. However, this stroma transcriptome differs substantially from that of osteoblastic lesions and reveals not only activation of pro-osteoclastogenic signals, but also interference with pro-osteoblastogenic factors. Thus, the osteolytic lesions seem to be not only the result of exaggerated bone resorption, but also of inhibition of bone formation. Importantly, the stem cell niche type and molecular components amplified are markedly different between osteoblastic and osteolytic lesions. This suggests different growth support requirements between osteinductive and pro-osteolytic cancer cells and, thus, the need for a differential therapeutic targeting aiming at interfering with tumour growth in osteoblastic and osteolytic lesions.

CABS1.3

Abstract not available

CABS1.4

The Bone Microenvironment and Myeloma

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Multiple myeloma (MM) is a plasma cell malignancy characterized by a tight relationship with the bone microenvironment cells. MM cells induce a significant alteration of the bone remodelling process due to the increase of osteoclast formation and activation and to the suppression of osteoblast differentiation leading to the impairment of bone formation and the development of osteolytic lesions. Recently an increase of osteocyte death has been also demonstrated in MM patients suggesting a potential role of these cells in the alterations of bone remodelling in MM. Interestingly, the increased osteoclastogenesis and the impaired bone formation in turn support myeloma cell proliferation and survival in vitro and promote tumoral progression in vivo. In addition it has been reported that quiescent myeloma cells with stem cell features may reside in the hypoxic osteoblastic niche for protection from apoptotic stimuli and are involved in the drug resistance and the relapse of the disease. Several studies have investigated the mechanisms involved in the relationship between myeloma cells and

bone microenvironment cells. MM cells are able to stimulate the osteoclastogenesis and to suppress osteoblast formation and viability either through the release of soluble factors or the cell-to-cell contact. Recently the potential role of *miRNAs* has been also suggested in the mechanisms involved in the suppression of osteoblast differentiation in MM. The identification of potential pathways involved in the relationship between bone and myeloma cells has leaded to identify new therapeutic targets including RANKL, Activin-A, Sclerostin, HIF-1α, and Wnt signaling pathways. Several new drugs have been developed and are under investigation for their future use in MM patients.

CABS 2.1

Radium-223 and Skeletal Metastases with Emphasis on the Osteoblastic Stroma

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Skeletal metastases are present in the vast majority of patients with castrate resistant prostate cancer (CRPC). The pronounced bone-tropism of disseminated tumour cells and the sclerotic phenotype of the metastases provide the basis for therapeutic use of bone-seeking radiopharmaceuticals (1). This lecture aims to discuss the mechanisms of action by which the bone-targeting alpha-emitter ^{223}Ra prolongs overall survival in patients with skeletal metastases form CRPC (2,3). Radium-223 in a chloride formulation deposits high-LET radiation within targeted sites following i.v injection (4). In a randomized Phase-2 trial, where 4 monthly injections of ^{223}Ra were given after external beam radiotherapy to the dominating painful site, a profound reduction of the alkaline phosphatase (ALP) bone-isoenzyme was observed (5). Also a significant decline in prostate specific antigen (PSA) was demonstrated with a survival benefit (5). This paved the way for the pivotal phase 3 ALSYMPCA study (2) that included 921 CRPC pts with bone mets (^{223}Ra, n=614; placebo, n=307). Here ^{223}Ra significantly improved overall survival vs placebo (median 14.0 vs 11.2 mo; HR=0.695; *P*=0.002) and was well tolerated. Radium-223 was in 2013 approved by EMA and FDA as the first-in-class alpha-emitter with a potent, targeted, anti-tumour effect on bone metastases and a favourable safety profile. In a post-hoc analysis (3), ^{223}Ra prolonged time to first symptomatic skeletal event versus placebo (median: 15·6 months *vs* 9·8 months, respectively; HR=0·66; 95% CI 0·52–0·83; p<0·001), and reduced both the risk of external beam radiation therapy for bone pain (HR=0·67; 95% CI 0·53–0·85) and spinal cord compression (HR=0·52; 95% CI 0·29–0·93). At the outset of the ^{223}Ra- development, the prevailing paradigm was that only the reactive zone surrounding the growing skeletal metastases (interphase between bone & cancer) expressed the osteoblastic stroma. In CRPC, however, there is a mesh of reactive stroma avid for ^{223}Ra entwined between cords of carcinoma cells (1) with ALP-expression by the CRPC cells per se; due to osteomimicry and epithelial-mesenchymal transition. This may impact on further clinical development of ^{223}Ra.

REFERENCES:

1. Sartor O, Hoskin P, Bruland OS: Targeted radio-nuclide therapy of skeletal metastases. *Cancer Treat Rev*, 39 (1), 18-26 (2012).
2. Parker C, Nilsson S, Heinrich D, *et al*: Alpha emitter radium-223 and survival in metastatic prostate cancer. *N Engl J Med,* 369 (3), 213-23 (2013).
3. Sartor O, Coleman R, Nilsson S, *et al*: Effect of radium-223 dichloride on symptomatic skeletal events in patients with castration-resistant prostate cancer and bone metastases: results from a phase 3, double-blind, randomised trial. *Lancet Oncol*, 15 (7), 738-46 (2014).
4. Bruland ØS, Nilsson S, Fisher DR *et al*: High-linear energy transfer irradiation targeted to skeletal metastases by the alpha-emitter 223Ra: adjuvant or alternative to conventional modalities? *Clin Cancer Res*, 12 (20), 6250s-6257s (2006).
5. Nilsson S, Franzén L, Parker C, *et al*: Bone-targeted radium-223 in symptomatic, hormone-refractory prostate cancer: a randomised, multicentre, placebo-controlled phase II study. *Lancet Oncol*, 8 (7), 587-94 (2007).

CABS 2.2

Preclinical Developments; Radium-223 in Osteolytic Bone Metastases and Osteosarcoma?

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In 2013, the United States Food and Drug Administration approved Radium-223 for the treatment of prostate cancer patients with bone metastases, based on the results of the ALSYMPCA trial. In doing so, Radium-223 became the first approved anticancer agent that extended overall survival in cancer patients not by directly targeting the tumor, but by targeting its (skeletal) metastases. Radium-223 may effectively target bone metastases in other tumors as well, such as breast and lung cancer. Furthermore, considering its mechanism of action, it is likely that Radium-223 will have antitumor activity in primary bone cancers such as osteosarcomas too. In this lecture, results of studies with Radium-223 in tumors other than prostate cancer will be discussed.

CABS 3.1

Mechanisms of Cancer Invasion and Metastasis: Potential Therapeutic Implications

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Bone The tumor microenvironment contributes to cancer invasion, growth and survival and thereby impacts tumor responses to therapy. We here developed an intravital infrared multiphoton imaging model for the multi-parameter visualization of collective cancer cell invasion, guidance by the tumor stroma, and short- and long-term resistance to experimental anti-cancer therapy. Using orthotopic fibrosarcoma and melanoma xenografts, we identify deep invasive growth driven by proliferation concurrent with collective invasion as main local invasion route, which further mediated resistance to high-dose hypofractionated radiation therapy (cumulative dose 20 to 40 Gy). This invasion-associated radioresistance niche comprised several hundreds of cells in close proximity to stromal structures, including collagen, basement vascular and myofibre membranes, and was able to re-establish tumor growth and relapse, thus escaping other imaging modalities but in vivo microscopy. Using simultaneous inhibition of β1

and β3 integrins by RNA interference or combined anti-β1/αV integrin antibody treatment, however, proliferation arrest, anoikis induction was achieved, ablating both tumor lesion and the resistance niche. Thus, the invasion niche represents a microenvironmentally privileged survival niche which provides integrin-dependent therapy resistance. To establish an model amenable to intravital multiphoton microscopy of bone metastases of prostate cancer (PCa), we implanted engineered humanized neobone into the mouse dermis. After *in vivo* implantation, TEBC maturation was monitored by μCT, MPM and histological analysis over time to generate a miniaturized-neobone with defined cortical thickness (50-60 mm) surrounding histologically mature murine bone marrow. PCa (PC3) lesions, after implantation into the bone cavity, were longitudinally monitored for growth, niche development and step-wise osteolysis, using multi-parameter recording of collagen/bone matrix, bone surface, blood vessels, stromal phagocytes and steoclasts, and PC3 cells. By combining innovative tissue engineering with optical windows, state-of-the-art fluorescence reporter technology and intravital MPM, this model will provide mechanistic and applied insight into the therapy response of bone metastases.

CABS 3.2

Abstract not available

CABS 3.3

CTCs – Who Cares? It is all about the DTCs!

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We have limited understanding of the relationship between CTCs and DTCs and to study these populations in clinical samples remains technologically challenging. CTCs are heterogeneous and the majority may not ultimately form metastases. So what will a detailed analysis of their genetic makeup really tell us? In addition, collection of DTCs most commonly involves bone marrow aspirates that potentially do not capture DTCs embedded in specific bone niches. Few studies have included collection and comparison of CTCs and DTCs from the same patient. The use of *in vivo* model systems combined with recent technological advances in cell labelling and imaging has provided new insights into the early stages of tumour cell dissemination to the skeleton. In particular, the role of specific microenvironments or niches, as well as how DTCs residing within these niches are affected by environmental signals and therapeutic targeting, is emerging. But to what extent can this information be translated to human disease? This lecture will provide a summary of the current understanding of CTCs and DTCs in development of metastatic disease and show the latest pre-clinical data from studies of DTCs in breast/prostate cancer. The role of CTCs and DTCs will be discussed; should CTCs be used as a measure of disease burden whereas DTCs should be the main therapeutic target? How useful is CTC gene expression data for patient outcome? Do CTC have to become DTCs in order to pose a risk?

CABS 4.1

Long Non-coding RNAs in Prostate Cancer Progression

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Current prostate cancer (PCa) biomarkers such as PSA are not optimal in distinguishing cancer from benign prostate diseases and predicting disease outcome. To discover additional biomarkers, we investigated PCa-specific expression of novel unannotated transcripts. Using Affymetrix Human Exon Arrays and RNA sequencing data, we identified 334 candidates referred to as EMC PCa-associated transcripts (EPCATs). These transcripts are uniquely expressed in subsets of PCa and not or barely expressed in normal prostate and other tissues. To validate their unique expression pattern, 15 EPCATs were validated by RT-PCR in cell lines and patient samples. Combined into a diagnostic panel, 11 EPCATs classified 80% of PCa samples correctly, while maintaining 100% specificity. High specificity was confirmed by *in situ* hybridization for EPCAT4R966 and EPCAT2F176 (SChLAP1) on extensive tissue microarrays. Besides being diagnostic, EPCAT2F176 and EPCAT4R966 showed significant association with pT-stage and were present in cancer precursor PIN lesions. We also found EPCAT2F176 and EPCAT2R709 to be associated with development of metastases and PCa-related death, and EPCAT2F176 to be enriched in lymph node metastases. Functional significance of expression of 9 EPCATs was investigated by siRNA transfection, revealing that knockdown of 5 different EPCATs impaired growth of LNCaP and 22RV1 PCa cells. Two EPCATs inhibited the migration of PC3 cells in a Boyden chamber assay. The EPCATs investigated so far do not exhibit a protein coding potential and are classified as long noncoding RNAs (lncRNAs). Ours and many other studies have now shown that our transcriptome consists of a limited number of coding RNAs (~22,000) and a huge variety of small and long noncoding transcripts. Surprisingly, many of these transcripts are uniquely expressed in one type of tissue or cancer. The observation that some of these lncRNAs are functionally relevant indicates that they do not just reflect random disease-related changes, but rather another layer of cellular regulatory complexity. Although the underlying transcriptional regulation is not fully understood, the novel PCa-associated transcripts are new diagnostic and prognostic markers with functional relevance to prostate cancer growth.

CABS 4.2

Abstract not available