FEATURES

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Three-Dimensional in Vitro Model to Study Osteobiology and Osteopathology 2715

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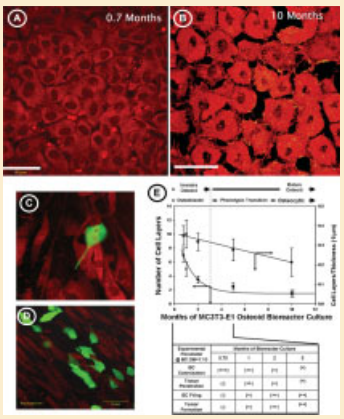

The bone is an amazing organ that grows and remodels itself over a lifetime. It is generally accepted that bone sculpting in response to stress and force is carried out by groups of cells contained within bone multicellular units that are coordinated to degrade existing bone and form new bone. Because of the nature of bone and the extensiveness of the skeleton, it is difficult to study bone remodeling in vivo. On the other hand, because the bone contains a complex environment of many cell types, is it possible to study bone remodeling in vitro? We propose that one can at minimum study the interaction between osteoblasts (bone formation) and osteoclasts (bone degradation) in a three dimensional (3D) "bioreactor". Furthermore, one can add bone degrading metastatic cancer cells, and study how they contribute to and take part in the bone degradation process. We have primarily cultured and differentiated MC3T3-E1 osteoblasts for long periods (2–10 months) before addition of bone marrow osteoclasts and/or metastatic (MDA-MB-231), metastasis suppressed (MDA-MB-231BRMS1) or non-metastatic (MCF-7) breast cancer cells. In the co-culture of osteoblasts and osteoclasts there was clear evidence of matrix degradation. Loss of matrix was also evident after co-culture with metastatic breast cancer cells. Tri–culture permitted an evaluation of the interaction of the three cell types. The 3D system holds promise for further studies of cancer dormancy, hormone, and cytokine effects and matrix manipulation.

Color-Coded Imaging of Breast Cancer Metastatic Niche Formation in Nude Mice 2730

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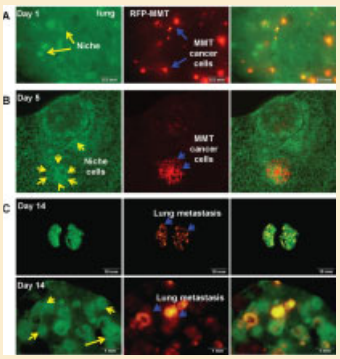

We report here a color-coded imaging model in which metastatic niches in the lung and liver of breast cancer can be identified. The transgenic green fluorescent protein (GFP)-expressing nude mouse was used as the host. The GFP nude mouse expresses GFP in all organs. However, GFP expression is dim in the liver parenchymal cells. Mouse mammary tumor cells (MMT 060562) (MMT), expressing red fluorescent protein (RFP), were injected in the tail vein of GFP nude mice to produce experimental lung metastasis and in the spleen of GFP nude mice to establish a liver metastasis model. Niche formation in the lung and liver metastasis was observed using very high resolution imaging systems. In the lung, GFP host-mouse cells accumulated around as few as a single MMT-RFP cell. In addition, GFP host cells were observed to form circle-shaped niches in the lung even without RFP cancer cells, which was possibly a niche in which future metastasis could be formed. In the liver, as with the lung, GFP host cells could form circle-shaped niches. Liver and lung metastases were removed surgically and cultured in vitro. MMT-RFP cells and GFP host cells resembling cancer-associated fibroblasts (CAFs) were observed interacting, suggesting that CAFs could serve as a metastatic niche.

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Localization of α-Dystrobrevin in Cajal Bodies and Nucleoli: A New Role for α-Dystrobrevin in the Structure/Stability of the Nucleolus

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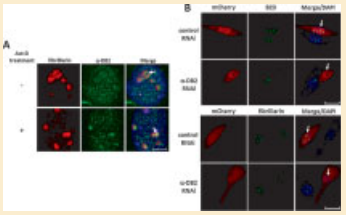

α-Dystrobrevin (α-DB) is a cytoplasmic component of the dystrophin-associated complex involved in cell signaling; however, its recently revealed nuclear localization implies a role for this protein in the nucleus. Consistent with this, we demonstrated, in a previous work that α-DB1 isoform associates with the nuclear lamin to maintain nuclei morphology. In this study, we show the distribution of the α-DB2 isoform in different subnuclear compartments of N1E115 neuronal cells, including nucleoli and Cajal bodies, where it colocalizes with B23/nucleophosmin and Nopp140 and with coilin, respectively. Recovery in a pure nucleoli fraction undoubtedly confirms the presence of α-DB2 in the nucleolus. α-DB2 redistributes in a similar fashion to that of fibrillarin and Nopp140 upon actinomycin-mediated disruption of nucleoli and to that of coilin after disorganization of Cajal bodies through ultraviolet-irradiation, with relocalization of the proteins to the corresponding reassembled structures after cessation of the insults, which implies α-DB2 in the plasticity of these nuclear bodies. That localization of α-DB2 in the nucleolus is physiologically relevant is demonstrated by the fact that downregulation of α-DB2 resulted in both altered nucleoli structure and decreased levels of B23/nucleophosmin, fibrillarin, and Nopp140. Since α-DB2 interacts with B23/nucleophosmin and overexpression of the latter protein favors nucleolar accumulation of α-DB2, it appears that targeting of α-DB2 to the nucleolus is dependent on B23/nucleophosmin. In conclusion, we show for the first time localization of α-DB2 in nucleoli and Cajal bodies and provide evidence that α-DB2 is involved in the structure of nucleoli and might modulate nucleolar functions.

Oxygen Sensitivity of Placental Trophoblast Connexins 43 and 46: A Role in Preeclampsia?

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Several gap junction connexins have been shown to be essential for appropriate placental development and function. It is known that the expression and distribution of connexins change in response to environmental oxygen levels. The placenta develops under various oxygen levels, beginning at a low oxygen tension of approximately 2% and increasing to a tension of 8% after the onset of the uteroplacental circulation. Moreover, it has been shown that during preeclampsia (PE) placentas are subjected to chronic hypoxia. Therefore, we investigated oxygen sensitivity of placental connexins 43 and 46. Using the trophoblast cell line Jar, we demonstrated that the expression of connexin43 increased during acute hypoxia but decreased during chronic hypoxia. Chronic hypoxia resulted in the translocation of connexin43 from the membrane to the cytoplasm and in a reduction in its communication properties. In contrast, the expression of connexin46 was down-regulated during chronic hypoxia and was translocated from perinuclear areas to the cell membrane. Hypoxia-inducible factor (HIF) knockdown showed that the translocation of connexin43 but not that of connexin46 was HIF-2α dependent and was mediated by phosphoinositide 3-kinase. The up-regulation of connexin43 in combination with the down-regulation of connexin46 was confirmed in placental explants cultivated under low oxygen and in placentas with early-onset PE. Taken together, in Jar cells, placental connexins 43 and 46 are regulated during periods of low oxygen in opposite manners. The oxygen sensing of connexins in the trophoblast may play a role in physiological and pathophysiological oxygen conditions and thus may contribute to PE.