Transplanted Bone marrow cells engraft within the mouse epidermis and proliferate with no evidence of cell fusion

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Introduction. Bone marrow (BM) cells engraft in non-haematopoietic tissues and form adult cell lineages. We investigated the contribution of BM to epidermal regeneration. Method. Lethally-irradiated female mice were rescued by BM transplant from GFP male mice. Epidermal wounding was induced 6 weeks post-transplant. Epidermis was harvested 4, 7 and 30 days post-wounding, or keratinocytes were isolated from the epidermis and re-suspended in growth medium. BrDU was injected 2 hours before sacrifice. Donor cells were detected by GFP immunohistochemistry (IHC), or *in situ* hybridisation (ISH) for Y chromosome, combined with IHC. For fusion studies, a similar model was employed using male wild type (WT) recipients. Results. BM contributes to 7.2% of keratinocytes in normal epidermis, increasing significantly to 11.5% in wounded epidermis. BMDKs were present in clusters in the regenerating epidermis. BMDKs frequently engrafted epidermal stem cell zones and often expressed CD34, an epidermal stem cell marker. In the epidermis, BM-derived cells, morphologically typical of keratinocytes, were negative for macrophage marker F4/80, and neutrophil marker, Ly6G. Fluorescent IHC for GFP, keratin-14 (k14), and BrDU showed that BMDKs proliferate: supported by the presence of GFP+, k14+ keratinocyte *in vitro* colonies from WT epidermis transplanted with GFP+ BM. ISH for Y chromosome combined with GFP and k14 IHC in epidermis from male WT mice transplanted with male GFP+ BM, showed that GFP+ cells express k14, but do not fuse with pre-existing keratinocytes. Conclusion. We demonstrate for the first time, BMDKs capable of proliferation *in vivo*, without fusion with native keratinocytes.

Myofibroblasts Associated with Liver Tumours are Frequently of Bone Marrow Origin

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Aim: Myofibroblasts produce the tumour capsule seen around certain liver cancers and may have a pro-angiogenic role in liver metastasis. We have recently found that a significant proportion of hepatic myofibroblasts are of bone marrow (BM) origin in human liver fibrosis. Our aim was to identify whether myofibroblasts associated with liver tumours had a BM origin in a murine model of chronic liver injury and hepatocellular carcinoma.
Methods: Hepatitis B surface antigen transgenic female mice (HBsAg-tg) received lethal irradiation followed by a BM transplant with whole or lineage-depleted (Lin⁻) BM from a wild-type male donor. Prior to transplantation the BM cells were transduced with a HIV vector carrying the GFP marker gene under the control of a spleen focus forming virus (SFFV) promoter. After 6 weeks mice were treated with retrorsine to block hepatocyte regeneration. After this, half of them received splenocytes from females immunized with HBsAg DNA plasmid. After 6 months the animals were sacrificed and BM derived cells were tracked using *in-situ* hybridisation (ISH) for the Y chromosome and immunohistochemistry for GFP.
Results: Tumours were seen within the livers. The hepatocytes within the liver cancers were not BM derived. There were numerous Y chromosome positive myofibroblasts. Smooth muscle cells within large vessel walls were frequently of BM origin.
Conclusions: Many myofibroblasts associated with liver tumours are of BM origin in this model. Both the whole and Lin⁻ fraction of BM contains cells with a myofibroblast potential. These circulating cells may have a role in the pathogenesis of liver tumour development.

β-Dystroglycan Is Constitutively Expressed At The Intercellular Junctions of Epithelial Cells And This Expression Is Frequently Absent In Common Cancers

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Dystroglycan is a protein with extracellular α and transmembrane β subunits which link the extracellular matrix and cytoskeleton by binding to laminin and other matrix molecules. Previously published studies have shown reduced expression of α-dystroglycan in many cancers but β-dystroglycan has only been investigated in a few cases of prostate, breast and oral cancer. We have performed an immunohistochemical survey of β-dystroglycan expression on custom made tissue arrays containing 389 tissue cores representing 29 human tissue types and 23 different tumour types. Immunohistochemistry for dystroglycan was performed using a monoclonal antibody raised against the cytoplasmic domain of β-dystroglycan. In normal glandular and transitional epithelium there was strong dystroglycan expression at the intercellular junction between the epithelial cells, and between epithelial cells and the basement membrane. In squamous epithelium there was strong intercellular staining in the basal epithelial layers in skin and cervix but this was absent in the oesophagus. β-dystroglycan was completely absent or very weakly expressed in the majority of cancers including 98% of colorectal cancers (81 cases), 100% of ureteric transitional cell cancers (57 cases), 100% of oesophageal cancers (10 squamous, 10 adenocarcinoma) but it was present in some malignant tumours including cutaneous basal cell cancers. This is the first comprehensive survey of β-dystroglycan expression in human tissues and cancers. The absence of this important transmembrane protein in the majority of cancers may play a significant role in tumour progression and the mechanisms for this require further investigation.

Markers Of Adenocarcinoma Characteristic Of The Site Of Origin – Development Of A Diagnostic Algorithm

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BACKGROUND: Patients with metastatic adenocarcinoma of unknown origin are a common clinical problem. Knowledge of the primary site is important for their management, but histologically such tumours appear similar. Better diagnostic markers are needed to enable the assignment of metastases to likely sites of origin on pathological samples.
METHODS: Expression profiling of 27 candidate markers was performed using tissue microarrays and immunohistochemistry. In the first round, we studied 352 primary adenocarcinomas, from seven main sites (breast, colon, lung, ovary, pancreas, prostate and stomach) and their differential diagnoses. Data were analysed in Microsoft® Access and the Rosetta system and used to develop a classification scheme. In the second round, we studied 100 primary adenocarcinomas and 30 paired metastases.
RESULTS: In the first round, we generated expression profiles for all 27 candidate markers in each of the seven main primary sites. Data analysis led to a simplified diagnostic panel and decision tree containing 10 markers only: CA125, CDX2, CK7, CK20, ER, GCDFP-15, lysozyme, mesothelin, PSA and TTF1. Applying the panel and tree to the original data provided correct classification in 88%. The 10 markers and diagnostic algorithm were then tested in a second, independent, set of primary and metastatic tumours and again 88% were correctly classified.
CONCLUSIONS: This classification scheme should enable better prediction on biopsy material of the primary site in patients with metastatic adenocarcinoma of unknown origin, leading to improved management and therapy.