



Lincoln's Inn Fields

Haematopoietic Stem Cell

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The laboratory is interested in purifying normal and leukaemic human stem cells using the non-obese diabetic/severe combined immunodeficient (NOD/SCID) xenotransplantation as a read out assay. Comparison of the phenotype, and molecular pathways regulating especially self-renewal between the two compartments might help develop new therapeutic strategies aiming at more specifically killing the leukaemia stem cell (LSC) pool. Other projects are aimed at dissecting the role of certain fusion proteins and gene mutations present in leukaemic patients in the leukaemogenic process.

Relationship between CD34^{neg} and CD34^{pos} cells

A few years ago, we and others identified a novel class of murine and human haematopoietic stem characterised by its lack of CD34 expression (Bhatia *et al.*, Nat. Med. 1998; 4: 1038-1040). One of our objectives is to study the relationship between CD34⁻ and CD34⁺ stem cells. We recently found using serial transplantation that CD34⁻ cells are more primitive than CD34⁺ cells. The CD34⁻ cells represent a quiescent reservoir of stem cells. We went on to

look at the molecular mechanisms to explain the quiescent of these CD34⁻ cells. We investigated the Notch, Wnt, TGFbeta pathways. We show that Nocth4 is highly expressed on these cells and that Delta4 expressed by placenta keep the cells in an undifferentiated stage and have no role in cell cycle control. We also find that the truncated form of Lef (Delta LEF) is highly expressed and that these cells do not respond to WNT3A *in vitro*. TGF beta is also highly expressed and we are at present studying the role of this pathway in the regulation of CD34⁻ cells (Fernando Anjos-Afonso *et al.*, Manuscript in Preparation).

Leukaemic stem cell

Immunodeficient mice are increasingly used to assay human hematopoietic repopulating cells as well as leukaemia initiating cells. One commonly used method to isolate these rare cells is to sort cells stained with fluorochrome-conjugated antibodies into fractions. The different fractions are then transplanted into immunodeficient mice to test their repopulating ability. The antibodies are generally treated as being neutral in terms of their effects on the experiment. Human repopulating cells are thought to express CD34 and lack CD38. Recently we have shown that anti-CD38 antibodies have a profound inhibitory effect on engraftment of cord blood and leukaemia cells. We show that this effect is Fc-mediated and can be overcome by treating mice with immunosuppressive antibodies. When this inhibitory effect is prevented, we demonstrate that the CD34⁺CD38⁺ fraction, of certain acute myeloid leukaemia samples, contains all or at least most leukaemia initiating cells capacity. We show using mice pre-treated with anti-CD122 that in some patients, LSCs could have a progenitor phenotype (CD34⁺/CD38⁺). Based on the heterogeneity of AML in terms of karyotype, differentiation stage of the blasts, and clinical outcome; it is not surprising that LSCs is more complex than previously

thought and can vary from patient to patient and also probably in the same patient depending on the stage of the disease. This heterogeneity not only indicates a potential differential origin or progression of the disease but also has important implications in the development of new therapies to eradicate these cells (Taussig *et al.* 2008).

Leukaemic stem cells and their microenvironment

Over the years, we have been trying to purify LSCs and test their repopulating activity using our xenotransplantation model. It appears that like normal HSC, leukaemia might be dependent on microenvironment clues. Thus, we have developed a way using two-photon microscopy to visualise and describe the location and nature of this niche. To our surprise, we show that both calvaria and long bones are highly vascularised and thus the notion of a separate vascular and osteoblastic niche should be revisited (Figure 1). Further studies are now aiming at describing the composition of the niche in both normal and leukaemic situation.

Stem cell from the stroma system of the bone marrow

A couple of years ago we prospectively characterised the mesenchymal stem cells (MSC) compartment present in mouse bone marrow. We found that these stroma cells are organised as a hierarchy and that a small fraction of these cells are capable of differentiation at least *in vitro* into seven different lineages (Anjos-Afonso F and Bonnet D. *Blood*, 2007; 109: 1298-1306). Because Oct4 and Nanog expression seem to be correlated with multilineage capacity we recently

investigated the role of Oct4 and Nanog in MSC development. Surprisingly, the overexpression of Oct 4 in human mesenchymal stroma cells induced cell death and senescence signals. This effect can be overcome by Nanog overexpression. Thus it appears that Oct.4 is not responsible for the multipotentiality of MSC and by itself act as an apoptotic/senescence signal (Bithiah Grace *et al.* Manuscript in Preparation).

Stem cells as a vehicle for gene and cell therapy

In collaboration with Dr Sam Janes, we evaluated the therapeutic potential of Bone marrow Derived Stem Cells (BMDSCs) and determined their ability to produce a sustained delivery of KGF to the lungs in an attempt to ameliorate pulmonary fibrosis through an increase in epithelial proliferation. For that purpose, we used the BLM-induced lung fibrosis model and the use of BMDSCs as therapy vehicles. We demonstrated, that this combined cell/gene therapy not only is able to deliver KGF to the injured lung parenchyma, but KGF protects from BLM-induced lung injury. We reported that KGF induces proliferation of alveolar type II cells and decrease pro-inflammatory cytokines and collagen levels within the lungs. Finally, comparing MSCs and HSCs as therapy vectors, our data suggests that HSCs are greater gene therapy vehicles able to ameliorate lung fibrosis (Susana Aguilar *et al.* Manuscript in Preparation).

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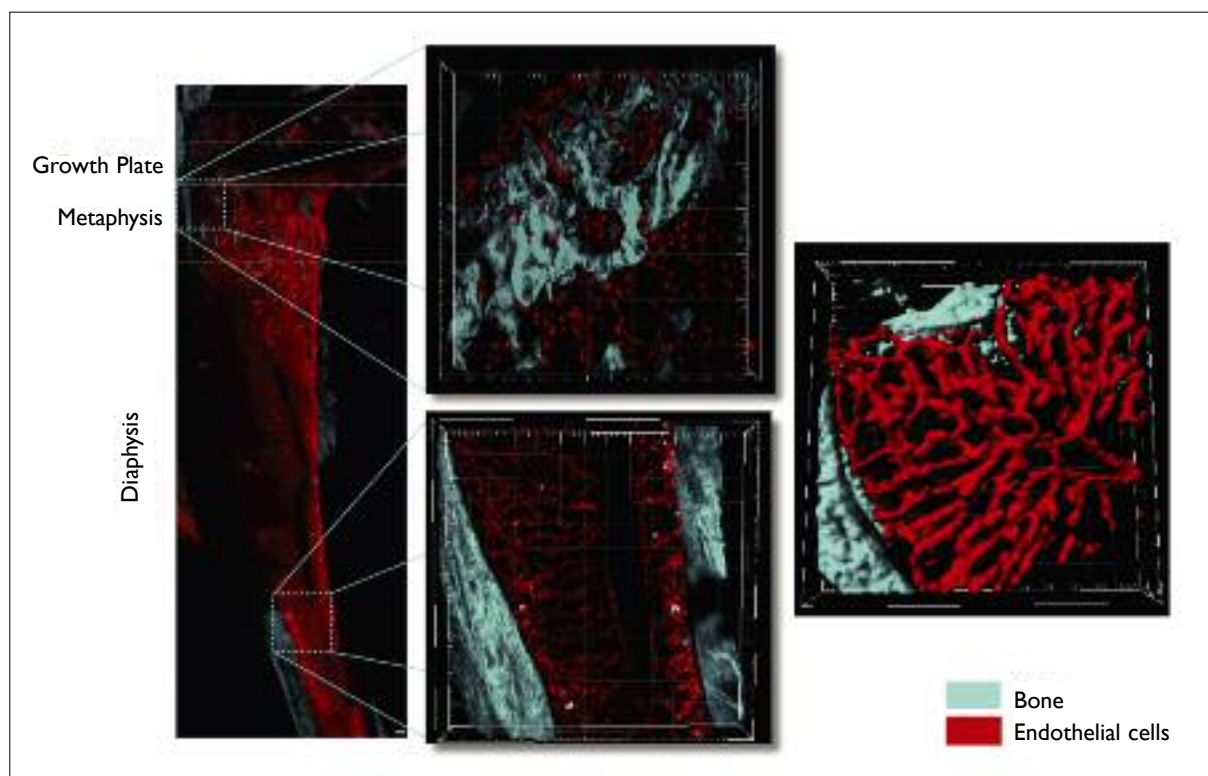


Figure 1. Vascularisation of long bone