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Multi-parameter isolation of primary lung cancer tumour stem cells (TSC); combining murine and human surface phenotyping in parallel with in-vivo tumour engraftment reveals multiple TSC phenotypes

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Introduction: There is emerging evidence that tumour comprises a hierarchy of biologically distinct cells. Tumour cells possessing the ability to regenerate growth have been termed tumour stem cells (TSC). TSC can be distinguished from non-tumourigenic population using surface phenotyping. The murine bronchoalveolar cancer stem cell has been defined as CD34+, 45-ve, 31-ve. CD133 had been reported as a TSC marker in human lung cancer. We postulate that there may be multiple cell surface markers defining TSC in lung cancer.

Methods: We prospectively collected surgical specimens from March 2007 to October 2008. Tumours are digested to single cells and then subjected to multi-parameter FACS phenotyping and/or xenograft formation assay in NOD-SCID or NOD-SCD IL2 gamma KO (NOG) mice. Xenograft formation indicating tumour-initiating cell activity is used as a surrogate for TSCs. Xenografts are serially passaged and subjected to further FACS assisted sorting to determine the surface phenotype of TSCs.

Results: Seven primary lung cancer specimens were collected; primary lung=61 (87%), LN=6 (9%), effusions=4 (6%), bone met=1 (1%). CD45+ haematopoietic cells comprises 55%±21 of all viable cells and CD45-ve cells comprises of 17%±11. After haematopoietic cells were excluded in phenotype analysis, the tumours cells can be fractioned to CD34+ 9.1%±12, CD133+ 11%±24, CD326+ 32%±27. Serially passaged tumours were re-digested and TSC activity determined by multi-parameter FACS sorting using CD133, CD34 and CD326 after exclusion of non-viable and murine cells. Of the xenografts produced (n=26), TSC activity does not rest exclusively in CD133+ fraction. Tumours can also arise from CD326+ and CD34+ fraction after concurrent negative gating for CD133.

Conclusion: We demonstrated the feasibility of high-resolution multi-parameter FACS analysis and sorting of primary lung cancer. Our data suggest the proportion of tumour cells within digests of primary lung cancers is no more than 20% of all viable cells. Our xenograft engraftment data suggest that human cells contribute to no more than 20% of all live cells obtained from xenograft experiments and CD133+ fraction is not the only one containing TSCs as previously reported in the literature.

Acknowledgement: This work was made possible by the STARR foundation US. I would also like to thank Drs M Moore and R Downey for their research support.

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Inactivation of Toll-like receptor 4 improves reendothelialisation in ApoE-deficient mice—impact of oxidative stress on endothelial progenitor cells

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Introduction: Atherosclerosis is an inflammatory disease which is in part mediated by Toll-like receptor 4 (TLR4). Endothelial injury, an initiating step in atherosclerosis, can be repaired through endothelial progenitor cell (EPC) activation, which has been shown to be diminished in patients with diabetes and/or vascular diseases. Vascular inflammation appears to impair the capacity of EPC in mediating the reendothelialisation process. It remains to be determined whether TLR4 is involved in this impairment.

Methods: ApoE-deficient (ApoEKO/TLR4WT) mice and ApoEKO mice lacking functional TLR4 (ApoEKO/TLR4KO) were used in this study. Wire injury was introduced to the right common carotid artery of the mice which were allowed to recover. Vascular repair was assessed by Evans blue staining of the injured arteries after 3 days. Circulating EPCs were quantified by flow cytometry analyses. Bone marrow–derived EPCs (BM-EPCs) were (1) assessed for reendothelialisation capacity after transplantation in vivo; (2) adhesion function in vitro; and (iii) reactive oxygen species production.

Results: (1) Reendothelialisation after wire injury was impaired in ApoEKO/TLR4WT mice and was associated with an increased number of circulating EPCs. Inactivation of TLR4 in ApoEKO/TLR4KO mice conferred an improved reendothelialisation, together with a paradoxical decrease in EPC number. Further findings suggested that the repair and adhesion capacity of EPCs from ApoEKO/TLR4WT mice was down-regulated. (2) Transplantation of BM-EPC isolated from ApoEKO/TLR4KO mice improved vascular repair, compared to cells from ApoEKO/TLR4WT mice. (3) Adhesion was diminished in BM-EPCs isolated from ApoEKO/TLR4WT mice and was normalised in BM-EPCs from ApoEKO/TLR4KO mice. (4) Oxidative stress was increased in BM-EPCs isolated from ApoEKO/TLR4WT mice, compared to those from ApoEKO/TLR4KO mice, suggesting a possible mechanism for EPC dysfunction.

Conclusion: Impaired reendothelialisation in atherosclerosis is attributable to a decline in EPC adhesion, which is reversible by TLR4 inactivation. Thus modulation of TLR4 activity may provide a mechanism to improve EPC function and hence protection against atherosclerosis.

Acknowledgement: This study was supported by a CRCG research grant, The University of Hong Kong, to FYL Li.