Leukemia (2007) 21, 2192–2235

www.nature.com/leu

LETTERS TO THE EDITOR

Splenectomy: a new treatment option for ALL tumors expressing *Hox-11* and a means to test the stem cell hypothesis of cancer in humans

Leukemia (2007) **21,** 2192–2194; doi:10.1038/sj.leu.2404927; published online 16 August 2007

In the cancer field, rare stem cells are thought essential to the onset, perpetuation and recurrences of human cancers. To date, cancer stem cells have been identified as causal in some malignancies, such as acute myelogenous leukemia, chronic myelogenous leukemias, B-cell lymphoblastic leukemias and possibly solid tumors.[1] The cancer stem cells based on this hypothesis are both necessary and sufficient for cancer growth and can be expanded in culture, unlike the cancer cells themselves. Therefore, an overriding goal in oncology has become the identification and eradication of the rare stem cells capable of sustained growth of the neoplasm—*in lieu* of the cancer itself. Ideally, those stem cells might be localizable in special reservoirs that would be targeted for therapeutics.

The strongest support for the cancer stem cell hypothesis has come from studies of acute myelogenous leukemia. A key study, for example, showed that only a small fraction of cells from human acute myelogenous leukemia, when transplanted into mice, managed to sustain tumor growth.[2] This xenotransplant study was consistent with the clinical success of bone marrow (BM) transplants for acute myelogenous leukemia based on the rationale that a few hematopoietic cancer stem cells in the marrow are responsible for tumor growth. Therefore, BM transplantation would likely decrease the burden of cancer stem cells with the BM transplant. However, a provocative article by Kelly and co-workers (2007) challenges the cancer stem cell hypothesis.[3] It questions the mouse xenotransplant study by arguing that few human cancer cells could survive transplantation into mice to promote tumor growth, not because of rare cancer stem cells, but because few human cancer cells could survive in the mouse milieu. A better murine test of the cancer stem cell hypothesis, according to the authors, would come from mouse to mouse transplants as opposed to xenotransplants (human cancer cells into mice). Indeed, when they undertook a study of mouse cancer cells of diverse lineages into mice, they found that diverse murine cancer cells survived the transplantation into another murine host and there induced growth of lymphoid and myeloid malignancies. The authors argued that the cancer stem cell hypothesis needed more rigorous testing in different models.

For almost a decade, our laboratory has focused on cellular therapies for diabetes and other disorders. But the findings we report here could be marshaled for another purpose, to pave the way for a rigorous test of the stem cell hypothesis of cancer—in humans. Our findings suggest that splenectomy, a low-risk surgical procedure to remove a nonessential organ, would be beneficial for certain patients with acute lymphocytic leukemia (ALL) of T-cell lineage, which aberrantly expressed *Hox11*. Splenectomy could immediately be tried not only as a test of the cancer stem cell hypothesis, but even more importantly as a therapeutic option for one subtype of ALL tumors: those expressing the highly conserved transcription factor *Hox 11*. To our knowledge, splenectomy as a treatment for *Hox11* expressing ALL tumors has not been performed.

We hypothesize that the source of *Hox11*-expressing ALL is in the spleen, based on our findings in an animal model[4] and our findings in humans, which are reported here. The spleen, but no other organs of *normal* adults, of all ages, holds a reservoir of stem cells that abundantly express the *Hox11* transcription factor. What is unusual about finding this reservoir in the spleen is that the prevailing dogma among developmental biologists is that *Hox 11* (also known as *Tlx-1*) is not expressed after birth. Developmental biologists have shown that, during embryogenesis, *Hox11* activates a cascade of genes controlling cell fate and differentiation, but ceases after birth.[5,6] A decade before investigations by developmental biologists, cancer biologists had identified *Hox 11* as a proto-oncogene in humans by virtue of its dysregulation or aberrant expression in certain types of T-cell ALL and in many forms of pediatric neural tumors.[7] *Hox 11* has prognostic importance in adults with T-cell ALL and offers a molecular approach to risk classification of adults with this disease. Thus far, the source of cancer stem cells for ALL subtype with *Hox11* expression has not yet been identified. It is clear, however, that the source is unlikely in the BM because clinical data suggest that the application of intensified BM transplants have not improved the rates of cure and instead have increased mortality. Like most cancers, ALL cells have limited proliferative capacity *in vitro*, suggesting that these cells are not the elusive progenitors *in vivo* that maintain the disease.

Here we report our investigation of whether the spleen of adult humans contains candidate stem cells with continuous expression of the *Hox11* oncogene. We obtained normal human spleens, produced during harvesting procedures for organ donation. The reasons for death were diverse (for example, automobile injury, cerebral hemorrhage, heart attack) but they qualified for organ donation. For these studies, frozen spleen tissue from 13 males and 17 females was processed in duplicate to prepare RNA from two separate tissue slices for reverse transcription (RT)-PCR analysis. In addition, control RNA was prepared from diverse donors with samplings of the BM, kidney, liver and tonsil samples from human donors. Tissue samples were procured from the National Disease Research Interchange (NDRI) and were of similar quality, consistently handled and stored prior to analysis.

Using RT-PCR analysis and DNA quantitative analysis, human spleen samples were prepared from 30 male and female spleen donors. The RT-PCR showed robust expression of *Hox11* between and within donor spleen samples. Multiple sampling regions of each human spleen were identified and processed separately. *Hox11* expression in the spleen was very apparent (Figure 1a). The *Hox11* expression in the human spleen was restricted to this organ and not other human lymphoid or non-lymphoid organs. Thus, *Hox11* expression is plentiful in the human spleen, as compared with negligible expression in BM, kidney, liver and tonsil (Figure 1a). The RT-PCR primer binds specifically to the *Hox11* gene as shown by the appearance of a

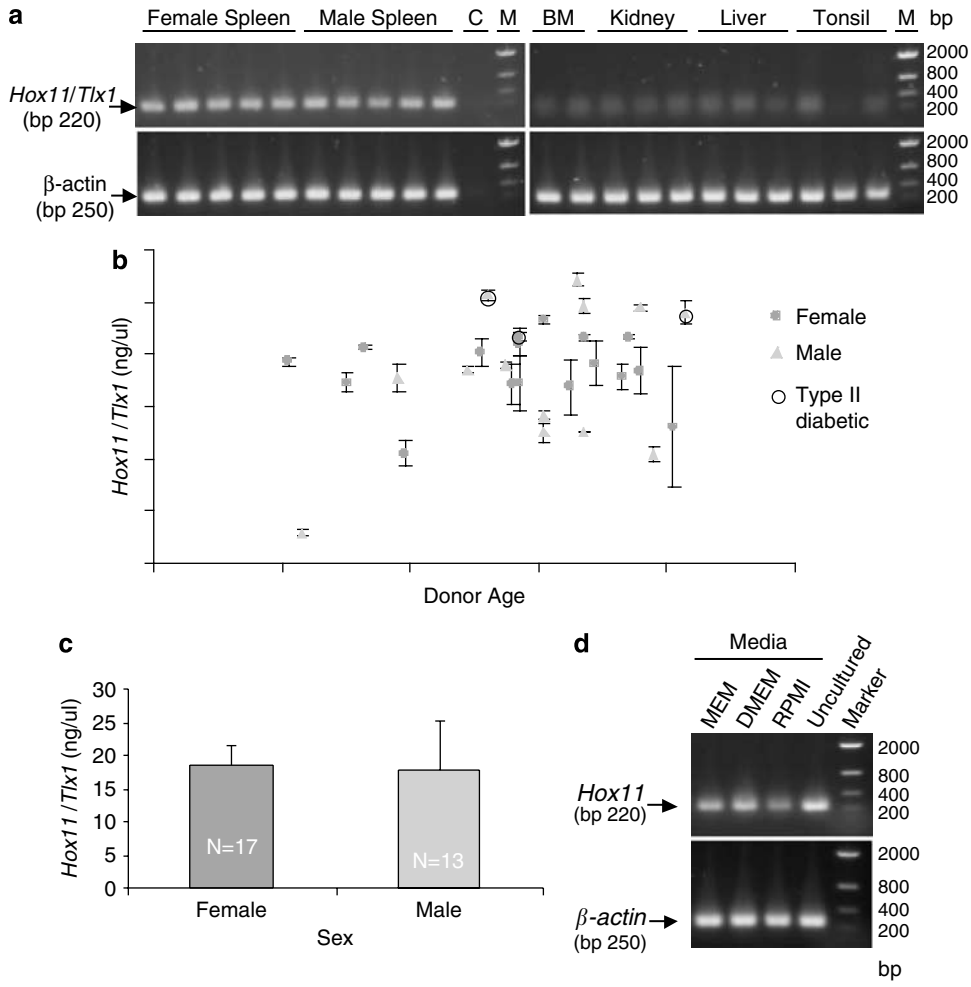

Figure 1 *Hox11* expression is unique to human spleen tissue, persists into adulthood; stem cell expansion with culture. (**a**) Abundant expression of *Hox11/Tlx1* in spleen of five female and five male donors. In contrast, bone marrow (BM), kidney, livers and tonsils from various donors showed relatively low expression of *Hox11* relative to that observed in human spleen. β-Actin for each sample confirms equal loading between samples and DNA markers (M) show that the *Hox11*-specific primer produce DNA products consistent with primer design (220 bp). A negative control (C) with primer and without mRNA template shows no measurable product. (**b**) Tissue expression of *Hox11* did not change by donor age in males or females. Error bars represent two independent observations from each donor. (**c**) There is no significant difference in *Hox11* expression level between male and female spleen donors. Error bars represent variable expression of all male and female donors. (**d**) Spleen cells expanded in culture to form a monolayer of attached cells over multiple passages for over 2 months showed the persistent expression of *Hox11* when grown in MEM, DMEM and RPMI media compared to uncultured spleen tissue. DMEM, Dulbecco's modified Eagle's medium; MEM, minimal essential medium.

single band of approximately 220 base pairs (bp) long and the lack of gene expression in a negative control (C) where no RNA template was added to the binding reaction. We observed no additional products formed at any other bp length as shown in the DNA retardation gel with DNA bp markers (M) with either the *Hox11* or β-*actin*-specific primer.

To determine if the level of *Hox11* expression in human spleen samples varied by age and gender of humans, we performed quantitative analysis of the DNA products formed by RT-PCR. Quantitative analysis was performed with Quant-iT PicoGreen dsDNA fluorescent probe from two independent tissue samples from the same donor. The level of gene expression in the spleen was consistently high within the same donor and between donors. Its expression remained high and did not vary by age (Figure 1b). We observed higher levels in patients with type II diabetes (Figure 1b). The three known diabetic donors had among the highest level of *Hox11* expression, consistent with a mouse literature suggesting insulin secreting beta cell precursor reside in the spleen and expand with select peripheral tissue damage. Quantitative analysis of *Hox11* spleen expression by gender showed no statistically significant gender difference (Figure 1c).

A trait of stem cells is expansion in tissue culture. Unlike differentiated or committed cell populations or even cancer cells, expansion in tissue culture is a unique trait of a precursor stem cell. Stem cells possess self-renewal and *Hox11* transfection itself can immortalize embryonic precursors and stem cells with partial hematopoietic potential.[8] Using isolated human spleen cells with enrichment by negative depletion of committed hematopoietic CD45 cell populations, we tested whether these human *Hox11*-positive cells show autonomous proliferation. We observed brisk proliferation and persistently abundant *Hox11* expression in tissue culture of the newly identified human stem cells of the spleen. Cell culture with expansion could be maintained beyond 2 months *in vitro* (Figure 1d). This suggests the potential for expansion of this unique population of *Hox11*-positive cells is maintained. To our knowledge, expression of *Hox11* has not been observed in any other human tissue

from normal adults and this study re-confirms this observation upon surveys of human tissues. Since expansions *in vitro* are a trait of stem cells, not differentiated hematopoietic or other differentiated cell populations, this human proliferative potential is consistent with the *Hox11* expression and a stem cell signature.

The observations that *Hox11* is abundantly and uniquely expressed in normal human spleen and its expression extends late into the aging process are of interest. First, the data suggest that the adult human spleen may have, similar to the mouse, a reservoir of well-regulated stem cells with multi-lineage potential. Second, the adult human spleen could also house the progenitor stem cells that, with transformation, could be a precursor for some forms of cancer such as *Hox11*-expressing T-ALL or pediatric neuronal tumors. Thus, our findings in normal humans could provide the rationale for splenectomy as a test of the cancer stem hypothesis in a certain patients with ALL. Finally, our findings suggest that splenectomy would be a low-risk therapeutic procedure for adults and pediatric patients with a poor prognosis and for whom there are few treatment options.

Acknowledgements

The Iacocca Foundation sponsored the funding for this report. The donation and retrieval of human spleens was made possible through the resources of the Human Data Biological Interchange. Dr Francisco Dieguez was supported in party by the NIH Endocrine Training Grant No. DK007028.

FJ Dieguez-Acuna[1], SP Gygi[2], M Davis[1] and DL Faustman[1]
[1]*Immunobiology Laboratories, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA and* [2]*Cell Biology, Harvard Medical School, Boston, MA, USA*
E-mail: faustman@helix.mgh.harvard.edu

References

1 Dalerba P, Cho RW, Clarke MF. Cancer stem cells: models and concepts. *Annu Rev Med* 2007; **58**: 267–284.
2 Hope KJ, Jim L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. *Nat Immunol* 2004; **5**: 738–743.
3 Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A. Tumor growth need not be driven by rare cancer stem cells. *Science* 2007; **317**: 337.
4 Kodama S, Davis M, Faustman DL. Diabetes and stem cell researchers turn to the lowly spleen. *Science SAGE* 2005; **2005**: pe2.
5 Raju K, Tang S, Dube ID, Kamel-Reid S, Bryce DM, Breitman ML. Characterization and developmental expression of Tlx-1, the murine homolog of HOX11. *Mech Dev* 1993; **44**: 51–64.
6 Dear TN, Colledge WH, Carlton MB, Lavenir I, Larson T, Smith AJ *et al.* The Hox11 gene is essential for cell survival during spleen development. *Development* 1995; **121**: 2909–2915.
7 Watt PM, Hoffmann K, Greene WK, Brake RL, Ford J, Kees UR. Specific alternative HOX11 transcripts are expressed in paediatric neural tumours and T-cell acute lymphoblastic leukaemia. *GENE* 2003; **323**: 89–99.
8 Keller G, Wall C, Fong AZ, Hawley TS, Hawley RG. Overexpression of HOX11 leads to immortalization of embryonic precursors with both primitive and definitive hematopoeitic potential. *Blood* 1998; **92**: 877–887.

Haematological malignancies in developing countries: is CML the commonest childhood leukaemia?

Leukemia (2007) **21,** 2194; doi:10.1038/sj.leu.2404880;
published online 2 August 2007

We would like to commend the editors for highlighting the usually silent issue of treatment differences in affluent *vs* developing societies[1] and stimulating discussion on such an important topic. We were, however, intrigued to read in the editorial that chronic myeloid leukaemia (CML) is the commonest childhood leukaemia in India rather than the 'usual' adult onset. The authors of this letter have worked in India, including one of the authors (CMP) for ~35 years, and have never seen CML as the most common type of leukaemia in children, which they believe has always been acute lymphoblastic leukaemia. We agree that the epidemiological literature on the incidence of haematological malignancies in India is sparse, but even in the reported literature, CML is not seen in children and has an epidemiology that is similar to that seen in the West.[2–4] We accept the fact that most government teaching hospitals come across numerous cases of chronic malignancies, where there is enough time for a patient to travel long distances, and a number of patients of acute leukaemia may be dying before receiving medical attention in rural areas. We are happy that the editors are optimistic on the treatment question but beg to differ that 80% of Indians cannot afford to pay for simple medicines, let alone an allogeneic transplant, which, even in the cost-constrained Indian scenario, would cost upwards of £3000, and further added costs of immunosuppression and treatment of infections. Busulphan and hydroxyurea still remain the treatment of choice for a majority of patients despite all the advances in CML. The Development of cheaper economic models of treatment, preferably 'single-shot' options, would be a major step forward.

AD Wechalekar[1] and CM Parande[2]
[1]*Department of Medicine, National Amyloidosis Centre, Royal Free and UCL Medical School, London, UK and* [2]*Department of Pathology, Lata Mangeshkar Institute of Medical Sciences, Nagpur, India*
E-mail: a.wechalekar@medsch.ucl.ac.uk

References

1 Gajewski JL, Robinson P. Do affluent societies have the only options for the best therapy? *Leukemia* 2007; **21**: 387–388.
2 Chaudhuri K, Sinha A, Hati GC, Karmakar R, Banerjee A. Childhood malignancies at BS Medical College: a ten year study. *Indian J Pathol Microbiol* 2003; **46**: 194–196.
3 D'Costa G, Siddiqui HM, Pradhan RM, Gupte SS. Pattern of leukemias: a ten-year incidence study of 242 cases.. *J Postgraduate Med* 1989; **35**: 191–195.
4 Kusumakumary P, Sankaranarayanan R, Padmakumary G, Varghese C, Kumar SR, Nair MK. Childhood cancer in Kerala, India. *Lancet* 1991; **338**: 455–456.