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JB Commentary

hDlk-1: a cell surface marker common to normal hepatic stem/progenitor cells and carcinomas

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Advances in stem cell biology have clarified that a tumour is a collection of heterogeneous cell populations, and that only a small fraction of tumour cells possesses the potential to self-renew. Delta-like 1 protein (Dlk-1) is a surface antigen present on foetal hepatic stem/progenitor cells but absent from mature hepatocytes in neonatal and adult rodent liver. Using a monoclonal antibody (mAb) against hDlk-1, Yanai *et al.* (Dlk-1, a cell surface antigen on foetal hepatic stem/progenitor cells, is expressed in hepatocellular, colon, pancreas and breast carcinomas at a high frequency. *J. Biochem.* 2010;148:85–92) have shown that human (h) Dlk-1 is expressed in human foetal, but not adult, liver and that 20% of all hepatocellular carcinomas (HCCs) are hDlk-1$^+$. Importantly, an even higher percentage of HCCs in younger patients are hDLK-1$^+$. These authors also found that hDlk-1 is present at high frequency in colon adenocarcinomas, pancreatic islet carcinomas and small cell lung carcinomas. Here, I discuss the implications of the expression of foetal hepatic stem/progenitor cell antigens on carcinoma cells.

Keywords: carcinoma/cell surface antigen/liver/monoclonal antibody/stem cell.

Abbreviations: AFP, alpha-feto protein; Dlk-1, delta-like 1 protein; EGF, epidermal growth factor; HCC, hepatocellular carcinoma; mAbs, monoclonal antibodies; NASH, non-alcoholic steatohepatitis.

Stem cells have the ability to both maintain proliferation (self-renewal) and differentiate into a variety of cell lineages (pluripotency), even after cell division. In addition to embryonic stem cells, there are tissue-specific populations of tissue (or somatic) stem cells in adult organs. Tissue stem cells supply new cells for individual organs and tissues to sustain their growth and maintain their activities. For example, hematopoietic stem cells in the bone marrow generate all lineages of blood cells, while neural stem cells differentiate into neurons and glial cells. When tissue stem cell functions are lost such that new cells are no longer supplied, various disorders of the vital organs can arise.

It has recently become clear that tumour cells are a heterogeneous population and that only a small fraction of them has the potential to self-renew (*1*, Fig. 1A). In this context, tumourigenesis can be considered a disease of unregulated self-renewal. The notion that a small number of cancer cells with the properties of stem cells (cancer stem/progenitor cells) are the origin of a tumour was originally proposed in the 1970s. However, the existence of these cancer stem/progenitor cells was difficult to prove experimentally. In the 1990s, developments in flow cytometry technology and a surge in our knowledge of cell surface markers made it possible to isolate and identify a single cell from a specific tissue. Studies of non-obese diabetic/severe combined immunodeficiency mice in 1997 revealed the existence of blood-specific cancer stem/progenitor cells in acute myeloid leukemia, followed in the 2000s by their isolation in a range of different cancers (*2*). However, although the expression of specific cell surface markers such as CD133 can be detected on cancer stem/progenitor cells in malignancies of the breast, brain and colon, the precise biological roles of these markers in these tumours are unclear (*3*). Thus, much work remains to be done on the identification and characterization of useful surface markers for cancer stem/progenitor cells.

In mammals, the foetal liver functions as a hematopoietic organ. In adults, the liver is less important for blood cell production but is essential for metabolism, detoxification, and the production of bile and serum proteins (*4*). The adult liver is a huge organ containing blood cells, fibroblasts and liver cells such as hepatocytes, biliary epithelial cells (cholangiocytes), liver sinusoidal endothelial cells and hepatic stellate cells. With respect to blood cells, more than 300 cell surface markers have been identified, and monoclonal antibodies (mAbs) have been raised against most of them. The use of these mAbs in conjunction with cell sorting has proved to be a powerful means of isolating and characterizing various blood cell subtypes. In contrast, very few liver-specific cell surface markers have been identified so that the properties of various liver cell subtypes have yet to be fully elucidated.

Foetal hepatic stem cells (hepatoblasts) are thought to be proliferative cells with the ability to differentiate into both hepatocytes and cholangiocytes (Fig. 1B). Hepatoblasts are derived from intestinal endoderm cells and form a hepatic bud that eventually develops into the foetal liver. In mice and rats, Delta-like 1 protein (Dlk-1), also known as preadipocyte factor 1 (Pref-1), is a transmembrane and secreted protein with epidermal growth factor (EGF)-like repeats (*5*). Dlk-1 is absent from neonatal and adult rodent liver but expressed on hepatoblasts. Dlk-1$^+$ cells isolated from foetal mouse livers form colonies containing cells of the hepatocyte or cholangiocyte lineages when cultured in the presence of hepatocyte growth

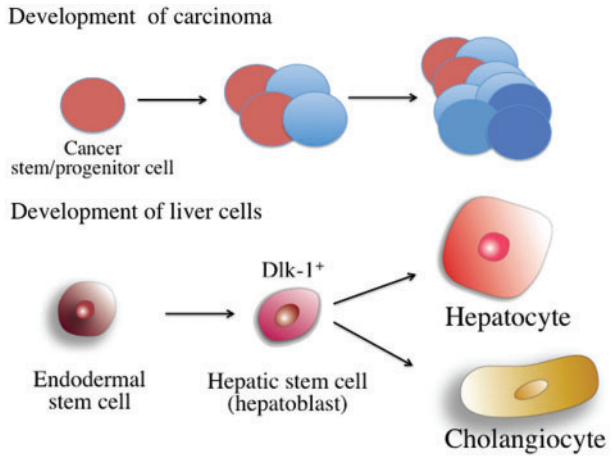

Fig. 1 Carcinogenesis and hepatic stem/progenitor cells.
(A) Proposed model of general carcinoma development. A cancer stem/progenitor cell proliferates and generates a heterogeneous cell population that contains additional cancer stem/progenitor cells (brown) as well as a variety of more differentiated tumour cells (shades of blue) that are not able to initiate tumourigenesis. (**B**) An endodermal stem cell gives rise to a hepatoblast (liver stem cell) that can differentiate into a hepatocyte or cholangiocyte. Hepatoblasts express Dlk-1.

factor and EGF. However, the precise physiological role of Dlk-1 in these situations is not clear. Using a mAb recognizing human (h) Dlk-1, Yanai *et al.* (*6*) showed that hDlk-1 is expressed in human foetal liver but not in adult liver. Significantly, 20% of all human hepatocellular carcinomas (HCCs) are positive for hDlk-1, with an even higher percentage of HCCs in younger patients being hDLK-1^{+}. These authors also demonstrated that hDlk-1 can be detected at high frequency in human colon adenocarcinomas, pancreatic islet carcinomas and small cell lung carcinomas.

Many HCCs (80–90%) occur in association with chronic hepatitis or cirrhosis of the liver. HCC develops in males and females at a ratio of 3:1 and occurs more often in Japan and east Asian countries than in Europe or North America. Recently, the relatively benign disease non-alcoholic steatohepatitis (NASH) has been found to be a precursor to liver cirrhosis and HCC in patients without detrimental drinking habits. Although alpha-feto protein (AFP) has been used as a tumour marker specific for HCC, it is often not expressed in an HCC when the tumour is still small and treatable. Thus, AFP is not useful for timely beneficial HCC diagnosis. If a marker for NASH could be identified, treatment could be instituted before progression to cirrhosis commenced. A similar difficulty exists for small cell lung cancers, which account for about 20% of lung tumours that are closely linked to smoking. No molecular marker specific for the early stages of this malignancy has yet been identified so that these high-grade carcinomas that easily make the transition to other organs are often discovered only when they are already advanced and impossible to remove. Molecular markers are also missing for islet cell tumours, including insulinomas, pancreatic islet carcinomas, glucagonomas and gastrinomas. Therefore, Yanai *et al.*'s discovery that hDlk-1 may be a molecular marker specific for the early stages of many of these tumours may lead to the development of valuable diagnostic tools and therapeutic agents.

Traditionally, pharmaceutical products are relatively low molecular weight compounds that are prepared by organic synthesis and administered orally. Although these low molecular weight drugs are clinically beneficial for many diseases, there are still a significant number of maladies for which they do not have a satisfactory therapeutic effect. Large molecular weight protein drugs were developed to address this gap, such as insulin for the treatment of diabetes, erythropoietin for anaemia and interferon for viral hepatitis. In the 1990s, the pharmaceutical industry was revolutionized by the development of therapeutic humanized mAbs as anti-cancer drugs. For example, rituximab, bevacizumab and trastuzumab are mAbs recognizing the human B cell surface antigen CD20, vascular endothelial growth factor and the growth factor receptor human EGF receptor-related 2, respectively (*7–9*). Yanai *et al.*'s work positions hDlk-1, a cell surface molecule of hepatic stem/progenitor cells, as a molecular marker of cancer stem/progenitor cells in several hard-to-detect malignancies. It may be possible to generate a diagnostic or therapeutic mAb targeting hDlk-1 that will identify early stage carcinomas and bring concrete clinical benefits to patients. Yanai *et al.*'s findings also imply that other cell surface molecules present on normal tissue stem/progenitor cells may emerge as useful markers of cancer stem/progenitor cells in a variety of tumours.

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Conflict of interest
None declared.

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