Blue Faery Bestows Two Awards to Fight Liver Cancer

Blue Faery: The Adrienne Wilson Liver Cancer Association is proud to announce the third annual Blue Faery Award (BFA) for Excellence in Liver Cancer Research. Primary liver cancer, also known as hepatocellular carcinoma (HCC), is the third leading cause of cancer deaths worldwide. Blue Faery created the award to recognise medical professionals who develop innovative research in the fight against HCC, which currently has no cure.

This year, for the first time, the BFA will go to two outstanding recipients. Dr. William B. Coleman, Director of Graduate Studies at University of North Carolina School of Medicine, researches pre-cancerous cells in the liver as a possible cause of HCC with the goal of targeting these cells before cancer starts. Dr. Hashem B. El-Serag, Chief of Gastroenterology and Hepatology at Baylor College of Medicine, focuses on public health awareness and early detection of the disease to give liver cancer patients the best chance of survival. Blue Faery feels with their different approaches to fighting liver cancer, Dr. Coleman and Dr. El-Serag are equally deserving of the BFA thus two separate awards will be given out.

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Liver Stem-like Cells: Progenitor Cells for Liver Cancer?

Primary liver cancer (primarily hepatocellular carcinoma – HCC) is a relatively rare neoplasm in the United States, but occurs at high incidence worldwide. In the United States, approximately 24,000 new cases were diagnosed and 19,000 cancer-related deaths occurred in 2010. In contrast, the disease represents the fifth most common cancer in men and the seventh most common cancer in women, worldwide: In 2008, there were 748,300 new cases worldwide, which equates to 5.9% of all cancers; with deaths reported at 695,900, i.e., 9.1% of all cancer deaths [1]. Prevalence varies greatly from world region to world region. High incidence rates are found throughout large portions of Asia and Africa, especially East and Southeast Asia and Central and Western Africa, with much lower incidence in Europe and the Americas. The highest incidence worldwide is among black males in Mozambique with 113 cases per 100,000 population. This incidence rate is > 500-fold higher than that for comparably aged white males in the United States and United Kingdom [2]. These statistics strongly suggest that factors related to genetic background and/or environmental exposure contribute significantly to incidence.

Numerous causative factors have been identified that contribute to liver cancer development, including viral infections, life-style factors (e.g., alcohol consumption) and exposure to naturally occurring carcinogens, industrial chemicals, pharmacologic agents and various pollutants [3]. The most well studied hepatocarcinogen is the natural chemical aflatoxin B1, produced by the *Aspergillus flavus* mold. This mold grows on rice or other grains (including corn) that are stored unrefrigerated in hot and humid parts of the world. Aflatoxin B1 is a potent, direct-acting liver carcinogen, and chronic exposure leads inevitably to cancer development. Numerous studies have shown a strong correlation between hepatitis B virus (HBV) infection and increased incidence [4] and more recently a strong association between chronic HCV infection and liver cancer has been recognised [5]. Whereas the occurrence of liver cancer in the United States has remained fairly low over the last 50 years, the incidence has been increasing dramatically, nearly doubling since 1997. The most likely cause of this trend is increased prevalence of chronic HCV infection.

Whereas the aetiology is clearly multi-factorial, liver cancer is usually associated with chronic hepatitis, with 60-80% of cases developing in cirrhotic livers, usually as the end result of chronic liver disease, irrespective of the causative agent involved [3]. The cirrhotic liver (which is pre-neoplastic) is characterised by scarring (related to connective tissue stroma collapse secondary to hepatocyte necrosis) and the presence of nodules of regenerating hepatocytes (Figure 1). HCC arises from the outgrowth of dysplastic hepatocytes in rare regenerative nodules (Figure 2).

Normal liver hepatocytes demonstrate very little cellular turnover. Hence, it is not surprising that the liver does not operate as a typical stem cell-fed lineage system like those of other self-renewing tissues (intestine, skin, bone marrow). However, there are multiple liver progenitor cell populations that have the potential to generate new hepatocytes [6,7]. These populations possess broad differentiation potential and function in the adult liver as "reserve" or "facultative" [8] stem-like progenitor cell compartments. They are activated to replicate and replace lost hepatocytes in certain forms of liver injury when the capacity of the remaining differentiated hepatocytes to proliferate is impaired. Recent evidence from experimental studies in rodents suggests there are at least two distinct cell populations that can be activated to generate new hepatocytes in liver injury: a population of normally quiescent, undifferentiated stem-like progenitor cells that reside in or around the portal tracts, which can be activated under certain pathological conditions to

> Identification of the molecular mechanisms that drive liver carcinogensis and development of therapeutic approaches that target these pathways will require a greater understanding of the various cells of origin of hepatocellular carcinoma
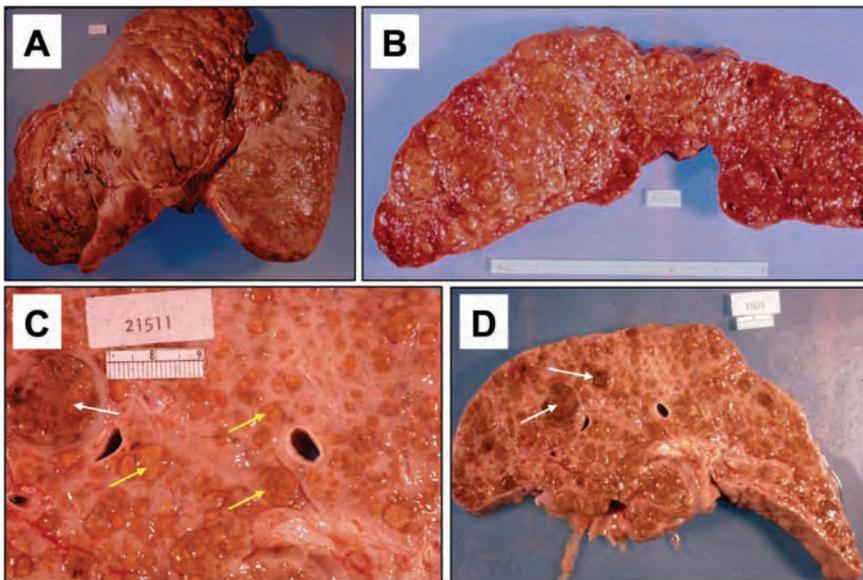
Figure 1: Primary hepatocellular carcinoma arises in the cirrhotic liver. (A) Autopsy specimen of liver heavily invested by primary hepatocellular carcinoma. (B) Cut section of the autopsy specimen reveals a large hepatocellular carcinoma (occupying the majority of the specimen). (C) Gross appearance of regenerative hepatocyte nodules (yellow arrows) in a cirrhotic liver containing hepatocellular carcinoma (white arrow). (D) The same cirrhotic liver showing multiple nodules of hepatocellular carcinoma (white arrows).
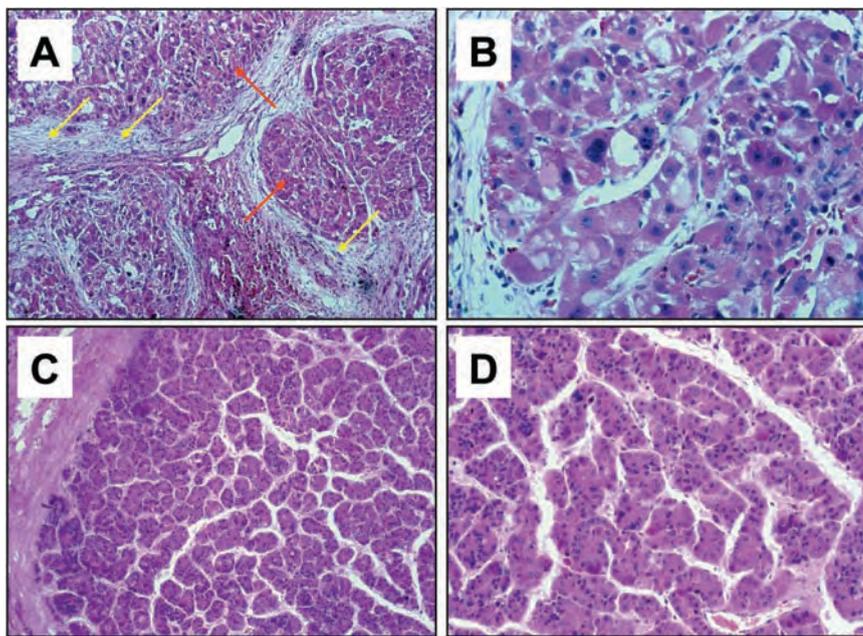

Figure 2: Microscopic appearance of cirrhotic liver and hepatocellular carcinoma. (A) Cirrhotic liver is characterised by fibrous connective tissue (yellow arrows) separating nodules of regenerative hepatocytes (red arrows). (B) Neoplastic cells composing hepatocellular carcinoma demonstrate nuclear and cellular pleomorphism. (C,D) Neoplastic hepatocytes of hepatocellular carcinoma for multi-cell thick plates in contrast to the structure of normal liver.

reestablish a proliferating-differentiating lineage (the oval cell reaction), capable of generating hepatocytes [9]; and a population of incompletely differentiated small hepatocyte-like progenitor cells (SHPC) that reside in the parenchyma and can be activated to replace lost hepatocytes [10]. Both of these reserve progenitor cell types could contribute to the origins of hepatocellular carcinoma.

We have extensively characterised SHPCs in the retrorsine-induced liver injury model [10-15]. Systemic exposure to retrorsine (a pyrrolizidine alkaloid) results in severe inhibition of the replicative capacity of fully-differentiated hepatocytes [10,16-18]. When confronted with a strong proliferative stimulus such as surgical partial hepatectomy (PH) [10,16,17] or hepatocellular necrosis [19], retrorsine-injured hepatocytes synthesise DNA but are unable to complete mitosis, and arrest as non-proliferative giant cells (megalocytes). In this model, neither retrorsine-injured, fully-differentiated hepatocytes nor oval cells proliferate sufficiently to contribute significantly to liver mass restoration after PH. Instead, the entire liver mass is reconstituted through a novel cellular response that is mediated by the emergence and rapid expansion of SHPCs, which share some phenotypic traits with fetal hepatoblasts, oval cells, and fully-differentiated hepatocytes, but are morphologically and phenotypically distinct [10]. Co-expression of hepatocyte markers and oval cell markers by early-appearing SHPCs suggest that these cells are not fully-differentiated, but display a phenotype similar to that expected for a cell type transitional between the bipotential hepatoblast (at embryonic day 14) and a fetal hepatocyte (at embryonic days 18-20).

To address the possible progenitor relationship between oval cells and SHPCs, retrorsine-exposed Fischer 344 rats were treated with the mitoinhibitory agent 2-acetamidofluorene (2-AAF) prior to PH . In marked contrast to animals treated only with retrorsine, the livers harvested from 2-AAF/retrorsine-treated rats at early time points after PH showed extensive proliferation of oval cells, but no proliferation of SHPCs [14]. Clusters of "small hepatocytes" were observed after PH, which continued to proliferate to restore normal liver mass several weeks after PH. Labeling of proliferating oval cells with bromodeoxyuridine after PH demonstrated that these "small hepatocytes" are progeny of oval cells and not an independent cell population [14]. Further evidence that "small hepatocytes" are not the same population as SHPCs was obtained by treating retrorsine-exposed rats with 2-AAF after PH; this resulted in blockade of SHPC expansion suggesting that the cells are susceptible to 2-AAF poisoning [14]. As the "small hepatocytes" observed in 2-AAF/retrorsine-exposed animals proliferate in the presence of 2-AAF, this is strong evidence that, although histologically similar, these cells are not SHPCs. Additional evidence that oval cells are not the source of SHPCs emerged from experimental models that employ the bile duct toxin diaminodiphenylmethane (DAPM) [15]. Here, retrorsine-exposed Fischer 344 rats were treated with DAPM prior to PH. While DAPM treatment resulted in significant bile duct damage, it had no effect on liver regeneration. Oval cells were never observed in DAPM/retrorsine treated animals but SHPCs were detected beginning early after PH. SHPCs continued to proliferate in a manner identical to that observed in animals treated with retrorsine only, until the normal liver mass was restored [10,15]. These results provide direct evidence that oval cell proliferation is not necessary for SHPC-mediated liver regeneration and provides the strongest evidence yet that these cells are not derived from oval cells. Instead, our observations suggest that the cell of origin of the SHPC is likely to be located in the liver parenchyma. However, the exact cell type giving rise to these cells remains unknown. Our group has posited that SHPCs may arise from a population of cells that are histologically similar to mature hepatocytes but phenotypically distinct [20]. This is

based on the original characterisation where it was demonstrated that SHPCs exhibit characteristics of fetal hepatoblasts, oval cells, as well as mature hepatocytes [10,11]. These characteristics suggest that a mature (fully-differentiated) hepatocyte is unlikely to be the source of a less differentiated cell population.

The cells of origin of liver cancer have long been debated in the field of liver carcinogenesis. Conventional wisdom holds that mature hepatocytes give rise to well-differentiated hepatocellular carcinoma, mature biliary epithelial cells give rise to biliary adenocarcinomas, and less differentiated cancers (or mixed cell type cancers) are derived from undifferentiated stem-like cells. SHPCs represent a novel candidate cell source for the genesis of hepatocellular carcinoma. Clearly this cell type could give rise to differentiated hepatocellular carcinomas, but may also contribute to the development of less differentiated cancers. Given the paucity of undifferentiated stem-like cell reaction in human liver injury and carcinogenesis and the inability of mature hepatocytes to respond to some forms of injury, it is likely that the SHPC will represent a key cellular target for hepatocellular carcinogenesis and therefore merits further investigation. For example, using the rat liver carcinogenesis model system, we can (i) combine carcinogen treatment with retrorsine injury to characterise the ability of small hepatocyte-like progenitor cells to generate liver cancers, (ii) characterise molecular alterations (e.g., changes in gene expression signature) that occur during liver carcinogenesis from this cell type in response to specific carcinogenic stimuli, (iii) evaluate SHPC contribution to spontaneous liver carcinogenesis, (iv) investigate approaches to liver cancer prevention in the high-risk patient, and (v) investigate new therapeutic approaches. The discovery and characterisation of the small hepatocyte-like progenitor cell population in rats will also enable important studies in human liver samples. These include utilisation of SHPC markers to identify the analogous cell type in normal or pathological human liver, investigation of SHPC contribution to liver regeneration in human liver preneoplasia (chronic hepatitis and cirrhosis) and examination of molecular pathways that control activation and proliferation of these cells in human liver, potentially enabling selective activation or targeted inhibition of proliferation.

The identification and characterisation of SHPCs in the rat liver model of injury and regeneration forms the foundation for the dawning of an exciting new chapter in liver carcinogenesis research. If SHPCs emerge as a major cellular target for neoplastic transformation, new research directions aimed at definitive identification of the molecular mechanisms that drive liver carcinogenesis and development of new therapeutic approaches that target these pathways can be pursued in a focused manner, accelerating discovery and leading more rapidly to reductions in human liver cancer incidence and mortality. ∎

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