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# Hepatic stem cells and their enzymatic markers- A review

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**ABSTRACT**

Stem cells are those cells which show capacity for self-renewal and ability to give rise to multiple differentiated cellular populations. Enzymatic activity, as a marker for cell proliferation and cell viability, is used by metabolic activity assays. Liver stem cells/progenitor cells can be a useful source of liver treatment. They can repopulate and restore injured liver. Fetal liver stem/ progenitor cells have been found to be more capable in this, but are subjected to ethical issues. Adult liver stem cells and stem cells from animals can be used. Alkaline phosphatase and lactate dehydrogenase are enzymatic markers of *in vitro* hepatocyte culture. During *in vitro* cell culture, in the culture medium, secreted alkaline phosphatase activity increases during exponential growth of cells, whereas low extracellular lactate dehydrogenase activity indicates increased number of viable cells. Alkaline phosphatase and lactate dehydrogenase activities can be used to assess hepatocytes proliferation *in vitro*.

**Key words**: Adult, Alkaline phosphatase, Fetal, Hepatic, Lactate dehydrogenase, Stem cells.

Stem cells are undifferentiated cells, defined by their ability to self-renew and to differentiate into mature progeny cells, which include both non-renewing progenitors and terminally differentiated effectors (Wagers *et al.,* 2002). Zech (2004) reported that there are four criteria to be fulfilled when defining a cell as a stem cell; first, the cells, which are able to undergo self-renewing, cell divisions. Second, daughter cells derived from a single stem cell and have the ability to differentiate into multiple cell types. Third, the cells those can functionally repopulate in a damaged tissue when transplanted. Fourth, the cells, which have the capacity to contribute differentiated progeny *in vivo*, even in the absence of tissue damage. The adult stem cells remained in a non-proliferative, quiescent state during most of their lifetime until stimulated by the signals triggered by tissue damage and remodeling. Current research stamps that adult stem cells can be a better resource in therapeutics as compared to embryonic stem cells since they are easy to harvest, express multi potency and low immunogenicity. Application of adult stem cells in the treatment of conditions like spinal injuries, tendon, cartilage and ligament defects, wounds and bone defects in animal models has been well described (Gade *et al.,* 2012).

The stem cells respond by proliferation and differentiation to replace senescent cells under normal circumstances or to restore destroyed tissues in pathological conditions. The epithelium of the skin is the classic example of the former. The stem cells, which are present in and around the damaged tissues, play a critical role in wound healing and tissue regeneration. The oval cells in liver represent a progeny of the hepatic stem cell compartment and at least in some instances to be a precursor for the hepatic tumors (Sell, 1990). The dying cells produce paracrine signals to activate the stem cells to start the tissue remodeling processes (Fang *et al.,* 2010). These properties make stem cells an attractive alternative to treat various degenerative disorders.

**Sources of stem cells:** Stem cells could be isolated from pre-implantation embryos, from different tissues of fetuses and adult organisms (Ratajczak *et al.,* 2008). Adult stem cells are available in many tissues such as brain, bone periosteum (Nakahara *et al.*, 1991), connective tissue (Young *et al.,* 2001), adipose tissue (Nakagami *et al.,* 2006), synovium (De Bari *et al.*, 2003), muscle (Jankowski *et al.*, 2002), amnion (In't Anker *et al.,* 2003), deciduous teeth (Yamada *et al.,* 2006), umbilical cord blood, blood vessels and blood (Lee *et al.*, 2004), dermal tissue (Chunmeng and Tianmin, 2004), placenta (Zhang *et al.*, 2005) and liver (Thorgeirsson, 1996). Anderson *et al.* (2001) classified stem cells into four broad types based on their origin, *viz.* stem cells from embryos (embryonic stem cells), stem cells from fetus (fetal stem cells), stem cells from umbilical cord (umbilical cord stem cells) and stem cells from adult (adult stem cells).

**Fetal and Adult Liver Stem Cells:** Michalopoulos and DeFrances (1997) stated that in adult life, liver is a quiescent organ, but when subjected to pathological aggressions, it responds with neoproliferation of mature hepatocytes and other liver cells to the point of complete organ regeneration. Overturf *et al.* (1997) reported that a fraction of adult mouse hepatocytes have the growth potential similar to that of

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hematopoietic stem cells. Using stem cells instead of organs in cell based therapy of liver diseases could permit (*i*) expansion of cells in an *in vitro* phase, (*ii*) genetic or immunological manipulation of cells for transplantation, (*iii*) tissue typing and cryopreservation of cells for transplantation in a cell bank, and (*iv*) the genetic modification of patient's own cells for autologous transplantation (Malhi and Gupta, 2001; Bianco and Robey, 2001).

Reports suggest that when fetal liver cells are introduced into an immunodeficient mouse, it expresses a repopulation potential ten times higher, when compared to cells isolated from the bone marrow of adult organisms (Taylor *et al.*, 2002). At the same time, various reports also indicate that based on the ability of proliferation and the capacity to differentiate into specific cell types, the hepatic progenitor/ stem cells (HPCs) from adult human liver may have potential therapeutic effects on end-stage liver failure (Theise, 2003). In addition, adult HPCs show a reduced risk of teratoma formation. The fetal liver is a rich source of stem cells in human (Rollini *et al.*, 2004). Fetal stem cells have certain advantages compared to adult stem cells. They are less immunogenic at lower differentiation stage and have a higher potential for repopulation and migration (O'Donoghue and Fisk, 2004). When human fetal liver cells are grown on collagen plates, they express the characteristics of multipotent progenitor cells flattened into a monolayer, which progressively increase in size and assume a polygonal morphology characteristic of hepatocytes (Dan *et al.*, 2006). Hristova *et al.* (2009) reported that fetal cells showed good proliferative activity and the isolated primary culture reached confluence approximately between 7 to 10 days after seeding. Darinskas *et al.* (2007) shown that after transplantation of human fetal liver stem cells into healthy mice, they exhibit homing effect in different organs *viz.* liver, brain, kidneys that led to the conclusion that they may substitute for injured cells in many tissues. Morini *et al.* (2010) reported that there is an early strong expansion of HPCs in patients received liver allograft with hepatocellular injury. Human hepatic stem cells, which are pluripotent precursors of hepatoblasts, hepatocytes and biliary epithelia, are located in ductal plates in fetal livers and in canals of Hering in adult livers (Schmelzer *et al.*, 2007). Fetal liver cells have good proliferative activity and capacity for long-term culture (Matsunaga *et al.*, 2008). Fetal stem cells have a certain immunomodulating effect when transplanted into an organ and expressed a unique set of cytokines and growth factors, which could stimulate the regeneration of the recipient tissue (Oertel and Shafritz, 2008). Clinical trials show improvement of patients with moderate forms of liver cirrhosis and hepatitis when treated with the transplantation of fetal liver cells (Pirogova and Pischkin, 2008). Jia and Zhou (2009) reproducibly achieved the isolation of mLEPCs (monkey liver epithelial progenitor cells) functioning as HPCs from a piece of liver tissue in normal adult rhesus monkeys. The purified mLEPCs displayed typical characteristics of HPCs, and differentiated into cholangiocytes and functional hepatocytes *in vitro*. Furthermore, mLEPCs repopulated and differentiated into hepatocytes in a liver injured mouse model.

Early fetal hepatocytes or hepatoblasts are the bipotential precursors and progenitors for both adult hepatocytes and bile duct epithelial cells (Grisham and Thorgeirsson, 1996). In the developing human liver, hepatic progenitor cells differentiate in steps marked by the acquisition or loss of specific phenotypic characteristics. It depends on the commitment of the progenitor cells to either hepatocyte or bile duct epithelial cell lineages that results in increased expression of one marker and loss of the other marker (Haruna *et al.*, 1996). These characteristics clearly identify bipotential hepatic progenitor cells. Dabeva *et al.* (2000) reported that 14-day old fetal rat liver contains lineage committed (unipotential) and uncommitted (bipotential) progenitor cells exerting different repopulating capacities, which are affected by the proliferative status of the recipient liver and the host site within the liver where the transplanted cells become engrafted. In the early stages of its development, the fetal liver consisted of hepatoblasts, which are precursors of hepatocytes and biliary cells (Schmelzer *et al.*, 2007; Walkup and Gerber, 2006) and haematopoietic cells, mostly erythroid progenitors (Isern *et al.*, 2008).

Reports clearly suggest that the proliferating ability of fetal and embryonic hepatic stem cells is higher as compared to adult hepatic stem cells. Sandhu *et al.* (2001) compared the repopulating capacity of embryonic day 14 fetal liver epithelial progenitor cells (FLEP cells) to adult hepatocytes, transplanted into normal liver. They found that the FLEP cells continued to proliferate upto six months after transplantation, whereas adult hepatocytes ceased proliferation within the first month. They also informed that both the number and size of clusters derived from FLEP cells gradually increased throughout time but such clusters decreased throughout time in case of transplanted mature hepatocytes. Therefore, isolation of progenitor cells from fetal organs is appropriate for establishing novel systems to investigate basic mechanisms and for cell/gene therapy (Malhi *et al.*, 2002). Hepatoblasts of the developing fetal liver give rise to parenchymal hepatocytes and biliary epithelial cells (Strain *et al.*, 2003). The hepatic progenitor cells are located in the most peripheral branches of the biliary tree, the ductules and canals of Hering (Roskams, 2006). Since use of human fetal liver is subjected to ethical issues and because of its unavailability for research studies, isolation of the stem cells from sheep is comparatively easy and capable of long term culture without differentiation. Further, the sheep fetal hepatic stem/progenitor cells show high proliferative capacity and exhibit characteristics similar

to human mesenchymal stem cells, which could be exploited instead of human material in research in order to avoid ethical restrictions (Hristova *et al*., 2009).

**Liver Enzymes as Markers of Hepatocytes Proliferation:** The normal rate of metabolism in tissues of the body is accompanied by a small release of enzymes from the cells and detected at low levels of activity in the blood plasma of normal animals. Higher levels of activity could occur when damage or hyperactivity of a tissue or organ results in more rapid leakage of enzymes from cells (Collis and Stark, 1977). Alkaline phosphatase and lactate dehydrogenase are the two enzymes that can be used as enzymatic markers of *in vitro* hepatocytes proliferation. These enzymatic markers form the part of metabolic assays of cell proliferation. Induction of DNA synthesis during hepatocytes replication is associated with increase of alkaline phosphatase activity (Emmelot and Bos, 1971). Sela and Sachs (1974) proposed that alkaline phosphatase might play role in regulation of DNA synthesis in cultured cells.

**Alkaline phosphatase (ALP):** Alkaline phosphatase is a zinc containing glycoprotein enzyme, which catalyzes the hydrolysis of phosphomonoesters at alkaline pH (Crofton, 1982; Sabellek, 1988). ALP is an "ectoenzyme" functioning in the external environment of the cell; this enzyme is anchored through a glycosyl-phosphatidyl inositol unit in the external cellular membrane (Calvo *et al*., 1996). Alkaline phosphatase activity found in the liver is arised in that organ and did not accumulate there from extrahepatic sites (Combes and Schenker, 1969). The alkaline phosphatase activity is 0.45 IU/g of liver weight of adult sheep (Keller, 1973), while ALP activity is 5 IU/g of wet weight of liver tissue in adult sheep (Clampitt and Hart, 1978). Collis and Stark (1977) reported that the liver alkaline phosphatase value is 0.3 IU/g wet weight of tissue in adult pig.

ALP is synthesized in a variety of tissues (Allen, 2003) and had different isoenzymes and isoforms (Sabellek, 1988). Different genes code different isoenzymes, whereas "isoforms" are products of the same gene with different posttranslational modifications (Magnusson *et al*., 1992). Four variants of ALP have been identified in domestic animals: bone ALP (BALP), which is synthesized by bone forming cells (osteoblasts), intestinal ALP, liver ALP (LALP), and in dogs, corticosteroid-induced ALP (CALP), which is predominantly produced by hepatocytes lining the bile canaliculi (Sanecki *et al*., 1987). LALP and BALP are products of the same gene and differ only in the number and location of posttranslational glycosylation sites, making them isoforms rather than isoenzymes of ALP; whereas CALP is a product of a different ALP gene, and therefore, is a true isoenzyme of ALP (Sanecki *et al*., 1990). At least three different structural gene loci are involved in the translation of isoenzymes of alkaline phosphatase. They are responsible for the bone, liver, kidney, intestinal and placental forms (Rodney and John, 1991). The alkaline phosphatase enzyme is present as a multimer with a molecular weight of 130–220 kDa. It is a tetramer when membrane-bound but circulates as a dimer (Price, 1993). ALP in dogs is found in many organs, including liver, bone, intestine, and kidney (Allen *et al.*, 1998; Hoffmann, 1988; Keller, 1981; Kidney and Jackson, 1988; Simon and Sutherland, 1977; Syakalima *et al.*, 1997; Syakalima and Takiguchi, 1998). Because of the very short half-lives of the intestinal and kidney isoenzymes, the only isoenzyme found in canine serum are the bone (B-ALP) and liver (L-ALP) isoenzymes and a unique isoenzyme called the corticosteroid-induced (C-ALP) isoenzyme (Kidney and Jackson, 1988; Syakalima and Takiguchi, 1998). The B-ALP isoenzyme originates from osteoblasts, and increased activity of this isoenzyme in serum is associated with conditions involving increased osteoblastic activity (Ehrhart *et al.*, 1998; Syakalima and Takiguchi, 1998). The L-ALP and the C-ALP isoenzymes are produced in the liver and are located on the hepatocyte plasma membranes lining the bile canaliculi and sinusoids (Solter and Hoffmann, 1995; Sanecki *et al.*, 1990; Syakalima and Takiguchi, 1998).

In the embryo, alkaline phosphatase activities are more widely distributed and all the tissues are rich in alkaline phosphatase levels at early stages of development (Moog, 1965). Miedema (1968) showed that alkaline phosphatase activity initially increased by 2 to 3 folds in cultures during pre-confluent proliferation and subsequently declined to near initial levels after confluency despite continued growth. Contact phenomena and crowding of the cells can be implicated in this regulation of alkaline phosphatase. Increased serum alkaline phosphatase levels found under different circumstances are due to increased synthesis of the enzyme by the liver (Jersky, 1974). Furthermore, in the absence of biliary obstruction, the enhanced production of alkaline phosphatase is accompanied by an increase in the excretion of the enzyme into bile. The stimulus for the enhanced production of alkaline phosphatase is phosphatidyl choline, a substance that is normally produced in the liver and excreted into the bile (Jersky, 1974).

When a rat hepatoma cell (R-Y121B) homogenate is incubated at 37°C, about 30 - 70 per cent of the total alkaline phosphatase is released into the supernatant fluid from the precipitate fractions. The release reached a plateau level after 10 h of incubation at 37°C (Kenji and Yosihiro, 1987). ALP activity also increases during cell cultures as observed in case of MDCK (Madin, Darby, canine, kidney cell line) cell monolayer, in which ALP activity increased by 37 fold from day second to eighth of culture, reaching a plateau at day 10 (Letellier *et al.*, 1987). Terryn *et al.* (2007) reported that alkaline phosphatase activity in seven day old primary PTC (proximal tubular cells) culture is about 20 times higher than in 14 day old primary cultures, indicating

the degree of differentiation is dependent on time in culture and decrease in ALP with time takes place due to differentiation. Alkaline phosphatase (ALP) activity measured in seven-day-old cultures of PTC (proximal tubule cells) is approximately 4 times higher than freshly isolated proximal tubule cells.

ALP alone is abnormal in a high proportion of breast cancer patients with bone metastases and/or liver metastases, and is more effective than AST and GGT in making a differential diagnosis (Crivellari *et al.,* 1995). In cancer patients, ALP is a sensitive indicator of mild biliary obstruction, thus being a very sensitive indicator of liver progression. Out of ALP, aspartate transaminase (AST) and *c*-glutamyltransferase (GGT), examined for their sensitivity in detecting cancer recurrence, ALP is the most sensitive one (Keshaviah *et al.,* 2007).

**Lactate dehydrogenase (LDH):** Lactate dehydrogenase is one of the so-called "soluble" glycolytic enzymes present in the cytoplasm of cells (Yoshiko and Stoward, 1997). Lactate dehydrogenase functions in the last step of glycolysis, catalyzing the reversible reduction of pyruvate to lactate. Singh and Kanungo (1968) reported that the lactate dehydrogenase of the supernatant fraction of liver from day old to 96 weeks of age of rats showed increase in enzyme levels as the age advances during the active growth phase, reached the maximum levels at 30 weeks of age and then declines. There is decreased activity of triose phosphate isomerase, glyceraldehyde phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceratemutase, and lactate/malate dehydrogenases in primary malignant hepatoma as compared to normal liver tissue (Balinsky *et al.*, 1973). Different reports suggest that the LDH activity is 64.26 IU/g of liver weight of adult sheep (Keller, 1973); while it is 27.6 IU/g, wet weight of tissue in adult pig (Collis and Stark, 1977). Clampitt and Hart (1978) recorded lactate dehydrogenase value of 100 IU/g of wet weight of liver tissue in adult sheep.

Leakage of cytoplasmic enzyme, lactate dehydrogenase (LDH) is commonly used as an index of hepatocellular damage and is applicable to both hepatocyte suspensions and monolayer cultures. Since LDH is released by hepatocytes in distress, it can be assayed during drug toxicologic studies as activity in either supernatant media or in cells in response to drug concentration and time of exposure (Tolman *et al.,* 1978; Anuforo *et al.,* 1978). The activity of lactate dehydrogenase in liver cells is about one third times to that of myocardial cells and considered as a possible enzyme test for the diagnostic use in liver diseases (Lott and Nemesanzky, 1986). The two fields in which lactate dehydrogenase and its isoenzymes can contribute to a major extent are in tumor diagnosis and liver transplantation. On suspicion of liver metastases the determination of lactate dehydrogenase and the isoenzymes are given a valuable contribution to the final diagnosis (Rotenberg *et al.,* 1989).

## CONCLUSION

Treatment of liver cancer and other liver ailments is still a major challenge. Liver stem cells can be a potential therapeutic material. Many studies are required in this field. Fetal liver stem/ progenitor cells have higher repopulation potential as compared to adult liver stem/progenitor cells, so they are better therapeutic material. However, fetal liver stem/progenitor cells are subjected to ethical issues. So, more studies are required to support better utilization of adult liver stem/progenitor cells in treatment of liver disorders. In addition, research should be done to find out the therapeutic potential of fetal liver stem/ progenitor cells available in animal species.. Alkaline phosphatase and lactate dehydrogenase can be used to study the hepatic proliferation, both *in vitro* and *in vivo*. In addition, they can be used to study the liver cells in cell cultures and in the animal, for making diagnosis about liver response to a treatment or hepatic neoplasia and metastases.

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