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Chemically Induced Hepatocellular Carcinoma and Stages of Development with Biochemical and Genetic Modulation: A Special Reference to Insulin-Like-Growth Factor II and Raf Gene Signaling

Biswajit Mukherjee*, Miltu Kumar Ghosh
and Chowdhury Mobaswar Hossain

*Department of Pharmaceutical Technology, Jadavpur University, Kolkata
India*

1. Introduction

1.1 Liver and its physiology

The liver, the largest organ in the body, predominantly functions as a biochemical laboratory where metabolism takes place. It has both endocrine and exocrine functions; and is also involved in numerous metabolic activities and acting as a storage depot. Once the nutritional substances and other chemicals such as drugs, carcinogens etc. reach in liver, they are metabolized by hepatic enzymes. The organ is located on the right side of the abdomen just beneath the diaphragm in human. Liver is a solid organ consisting of several lobes. Each lobe is constituted with numerous lobules which are in general hexagonal in shape (Figure 1). The center of each lobule is occupied by the central vein and the periphery of the lobule is delineated by a close arrangement of hepatic artery, portal vein, and bile duct; called "portal triads". The portal triads appear at the vertices of the hexagonal lobules. The vessels generated from the portal triads ramify and distribute along the sides of the lobule, and open into the sinusoids which have thin epithelial lining, a discontinuous layer of fenestrated endothelial cells. The liver has different types of cells. Oval cells are generally found near the portal triad. This rare cell-type has been claimed as hepatic stem cells by some researchers (Zamule et al., 2011). However, the major cell-type in liver is the polygonal hepatic parenchymal cells (hepatocytes). Hepatic lobules are made up of more than 80% hepatocytes which have an average size of 25 μ and occupy 70-90% of liver mass, depending on the species. They have clear cell membrane; sometimes with two nuclei. They have large deposits of glycogen, often with lipid droplets and basophilic materials. They also contain other cellular organelles such as mitochondria, rough endoplasmic reticulum (granular) and smooth endoplasmic reticulum (agranular), golgi apparatus, and lysosomes. The hepatocytes are arranged in stacks of anastomosing plates, separated by an anastomosing

* Corresponding Author

system of sinusoids. One or two cells thick hepatocytes appear radiating from central vein towards the periphery. They metabolize and excrete into sinusoids or bile canaliculi. They can undergo cell division to produce more hepatocytes. Other than the endothelial cells, the liver sinusoids contain phagocytic cells derived from monocytes, known as Kupffer cells. These macrophages phagocytize particulates and cell debris. Another hepatic cell-type is known as the ito cell. These are adipose or stellate cells.

Liver undertakes several important functions in our body. It

- produces bile which contains bile salts (sodium glycocholate, sodium taurocholate). The bile salts emulsify fats and oils and thus help in the digestion of them.
- involves in carbohydrate and fat metabolism, hemoglobin metabolism and lipid synthesis.
- stores many chemicals such as glycogens, vitamins, minerals and several metabolites.
- involves in detoxification and removal of many toxic chemicals, including drugs, carcinogens, and various toxins through bile from the body.
- converts circulating ammonia into urea by urea cycle (Ornithine cycle) and thereby reduces ammonia level in blood.
- produces serum proteins such as albumin, clotting factors.

1.2 Hepatic regenerative capability

Under normal condition in an adult, liver maintains its size. However, liver has the regenerative capacity. Under various stress conditions, liver-size may increase. Numerical

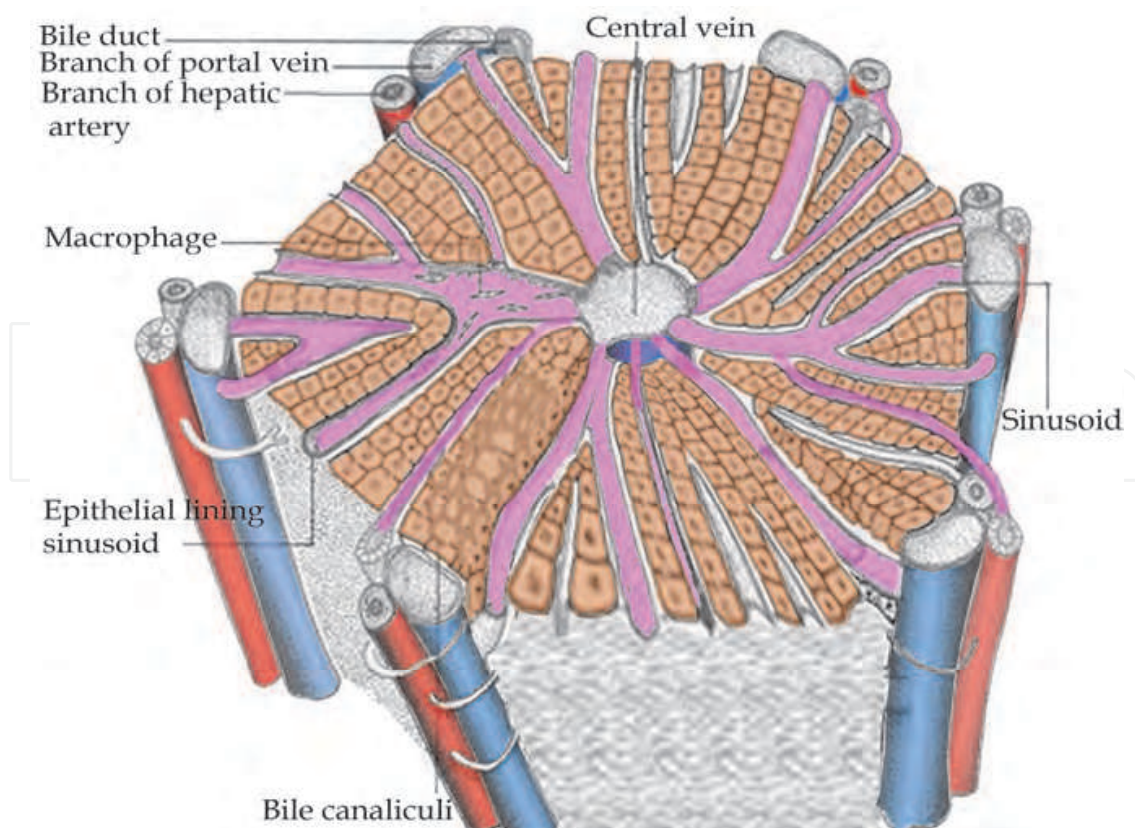


Fig. 1. Histological structure of a liver lobule

increase in hepatic cells by rapid cell division (hyperplasia) or increase in cell-size (hypertrophy) primarily causes enlargement of the tissue (Michalopoulos & DeFrances, 1997). Exposure to high levels of chemical toxicants causes increase in liver-size about 2-3 times of its normal size to combat the enhanced metabolic pressure exerted by chemical exposure (Michalopoulos & DeFrances, 1997). By regeneration process liver replaces the necrotic/ dead cells or the cells damaged due to toxicity. During hepatic continual regeneration process, the increased collagen synthesis and deposition result in fibrosis. This alongwith further continual hepatic cell damage due to various stress conditions cause liver cirrhosis (Lv et al., 2006). In cirrhosis, liver morphology alters. Scarring and nodularity appear. Normal hepatic function suffers and hepatic homeostasis decreases.

1.3 Liver cancer and its types

Cancer is the uncontrolled proliferation of cells that is caused by the multi-genetic defects. When the phenomenon occurs in liver cells, this is called hepatic cancer or liver cancer. The cancerous process begins in a few cells and the cells become immortal. They invade adjacent cells, intrude other nearby tissues (sometimes tissues at a distance) and metastasize. Uncontrolled proliferation of the cells causes solid mass formation in liver, resulting hepatic tumor. The predominant risk factors and etiological agents responsible for hepatocellular carcinoma in humans have been identified as chronic infection with hepatitis B virus or hepatitis C virus, exposure to aflatoxin B1 or other chemical carcinogens, and alcoholic cirrhosis and cirrhosis associated with genetic liver diseases.

1.3.1 Primary liver cancer

Primary liver cancer means that the cancerous process occurs and develops primarily in liver and does not start from the spread of cancerous cells located outside the liver. It is the most common type of hepatic cancer. There may be several causes of primary liver cancer. Chronic viral infections such as hepatitis B or C, some toxins, chemical induced hepatic damage, radiation-induced hepatic damage, and chronic liver diseases such as cirrhosis can cause primary liver cancer. Primary liver cancer has different types, too.

1.3.1.1 Hepatocellular carcinoma (also called hepatoma)

Hepatocellular carcinoma is the incidence of primary liver cancer in liver parenchyma cells or hepatocytes. People suffering from liver chronic diseases such as cirrhosis of liver are more prone to hepatocellular carcinoma. This is common to adult patients. However, in children and teenagers, similar pattern of the disease is called hepatoblastoma.

1.3.1.2 Cholangiocarcinoma

When primary liver cancer occurs in bile ducts it is called cholangiocarcinoma.

1.3.1.3 Angiosarcoma and hemangiosarcoma

These are fast growing rare type liver cancers.

1.3.1.4 Angiosarcoma

This rare form of rapidly growing fatal tumor develops in the endothelial cells of blood vessel of the liver.

1.3.1.5 Hemangiosarcoma

This is also developed from the lining of blood vessel, however, with relatively a slow speed. Blood-filled channels and spaces can be delineated under microscope. This is highly invasive type of cancer. It is commonly found in children. In patients suffering from hemangiosarcoma, the rupture of tumor leads to bleeding to death.

1.3.2 Secondary liver cancer

Malignant cells migrated from any tissues other than liver may invade hepatic tissue and develop neoplastic tumor in liver. This is described as secondary liver cancer. Spread of cancerous cells from outside the liver to the liver through blood flow or through the lymphatic system, the anchorage of the cells in liver, angiogenesis (formation of new blood vessels for supply of food and oxygen for new cells), and cellular proliferation leading to solid growth of mass are the possible sequences of secondary liver cancer. It is also called as metastatic cancer.

1.3.3 Experimental liver cancer in animals

Cancer in general is a multistage complex process by which uncontrolled proliferation of cells occurs. To understand the underlying mechanism in the process of development of the disease and its progress, it is important to develop the strategy to combat the dreadful disease. The need for the development of various in vivo cancer models has been in demand. Experimental liver cancer in animals is thus developed for studying the progress of the disease scientifically minutely in vivo and to develop therapeutic and other combating strategies to fight against it. There are various in vivo animal models already available for the purpose. Generally, virus-induced, radiation-induced, neoplastic cell-transplanted and chemical-induced liver cancer animal models have been widely studied.

2. Hepatocarcinogenesis

Hepatocarcinogenesis is the development of liver cancer due to the exposure of carcinogen (a chemical that produces cancer). Many hepatocarcinogens such as aflatoxins, acetylaminofluorene, diethylnitrosamine have been successfully used to develop hepatocarcinogenesis in animals. Experimentally, hepatocarcinogenesis is developed using different carcinogens and also in different animal species. Several genetic and epigenetic changes such as chromosomal deletions, rearrangements, aneuploidy, gene amplification, and mutations, formation of DNA adducts, DNA strand-break, modulation of DNA methylation, and modulation of cell signaling pathways, due to direct or indirect effect of carcinogen exposure lead to neoplastic transformation of hepatocytes in experimental animals. Hepatocarcinogenesis is a multistage complex process, which is preceded by early appearance of morphologically and genetically altered hepatic focal lesions, also known as preneoplastic lesions. Initially monoclonal populations of hepatocytes evolve primarily due to carcinogenic insult. These aberrant monoclonal populations of regenerative hepatocytes (focal lesions) develop hyperplastic nodules to dysplastic nodules, leading to hepatocellular carcinoma.

3. Hepatocellular carcinoma

The most common primary malignant tumor of liver is hepatocellular carcinoma. This primary liver cancer is also called hepatoma. As described above, liver has different types of cells such as hepatocytes, biliary cells, blood cells, Kupffer cells, Ito cells, perisinusoidal cells etc. However, about more than 80% of liver tissue consists of hepatocytes. The majority of primary liver cancer (>90%) arises from hepatocytes and is called hepatocellular carcinoma. During hepatocarcinogenesis, initial carcinogen insult results in initiated cells from normal liver parenchyma cells or hepatocytes by genetic alteration following an interaction generally with DNA. Subsequent tumor promotion by chronic exposure of carcinogen or a tumor promoter such as phenobarbital develop clonally selected expansions of initiated cell populations called hepatic altered foci by fixing the mutations for further genetic changes. Additional accumulations of genetic changes within these foci produce hyperplastic nodules that ultimately lead to the development of hepatocellular carcinoma.

3.1 Stages of hepatocellular carcinoma

Transformation of the initiated hepatocytes into hepatocellular carcinoma is a multistage complex process. Based on the various morphological (such as appearance, size, shape, growth) and biochemical (such as variation in staining patterns, and altered enzyme expression patterns) changes of these hepatocytes, leading to hepatocellular carcinoma, various stages of development of the disease, namely *initiation*, *promotion* and *progression*, have been described to understand the progress of the disease in a more defined way and to develop better therapeutic strategies.

3.1.1 Initiation

Exposure of genotoxic agents such as aflatoxins, 2-acetylaminofluorene, diethylnitrosamine, ionizing radiation etc. alters DNA sequence, causing mutations in the hepatocytes that

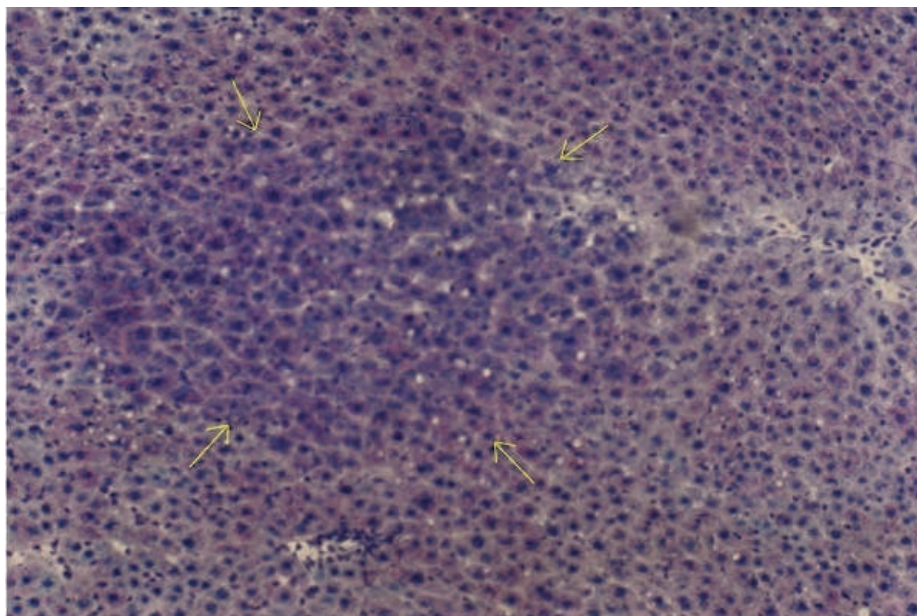


Fig. 2. Glycogen-stored early preneoplastic focal lesion (shown by yellow arrows) in rat hepatic tissue with Periodic Acid Schiff reaction. (Mukherjee et al. 2005)

develop potential to begin the transformation of normal cells to cancer cells. The mutations generally activate proto-oncogenes and/or inactivate tumor suppressor genes to develop hepatocellular carcinoma in the carcinogenic mechanism. The chemicals that cause the process (initiation) are called “initiators”. Initiation is an irreversible process for a small population of cells. It occurs with a single/ brief exposure to a carcinogen. Electrophilic moieties generated by genotoxic agents generally bind with DNA to form DNA adduct, hamper cellular DNA repair mechanism, and develop permanent DNA lesions. Thus, the normal cell becomes an initiated cell. The initiated cells can develop focal lesions (Figure 2), one or more of which can act as sites of origin for the subsequent development of malignant neoplasia (Farber & Sarma 1987).

3.1.2 Promotion

Initiated hepatocytes are unable to grow autonomously (Farber & Sarma 1987), although they gain the potential to favor proliferation by possessing alterations in gene and protein expressions. Upon exposure to an environment where initiated cells are at greater risk, further genetic alterations begin, causing some reversible changes in the initiated cell populations. Repeated or long-term exposure to a promoting agent (phenobarbital, dietary fat, ethanol, estrogens, chronic exposure of carcinogens) or by some processes (e.g., partial hepatectomy), or by physiological condition (e.g., the neonatal liver) or diseased liver (virus infected or cirrhotic liver), the initiated cells induce focal proliferations. It begins with a selective, clonal amplification of the initiated cells into focal proliferations.

3.1.3 Progression

One or more focal lesions from the promotion phase further proceed for more genetic and enzymatic alterations in the constituted hepatocytes, forming enzyme-altered foci. Such a lesion may form from a single lesion or by merging several lesions and may be large enough to form a macroscopic structure (hepatic nodule) (Figure 3) in the liver. They sometimes

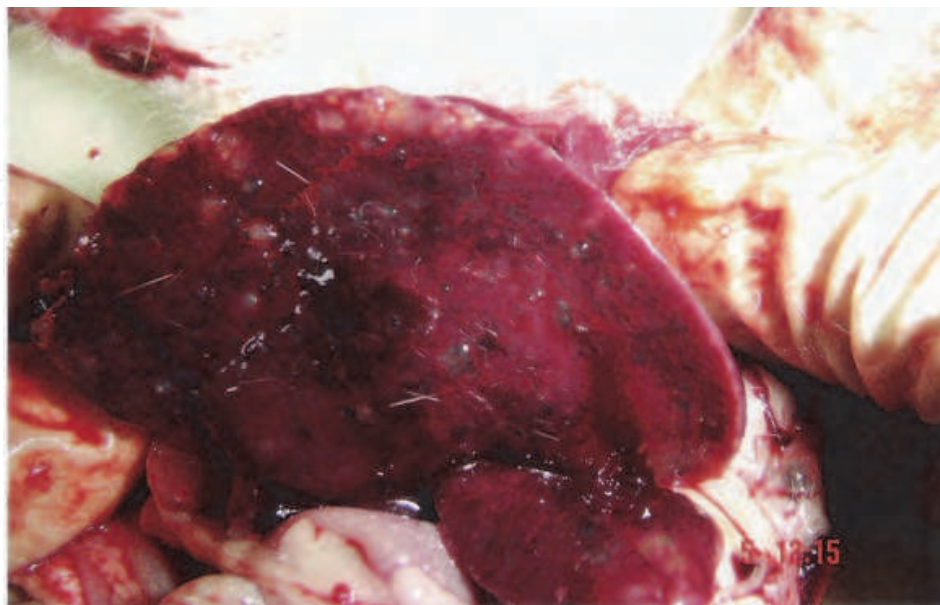


Fig. 3. An external morphology of liver tumor of a carcinogen-control rat showing multiple grayish-white and greenish-white hyperplastic nodules on the liver surface. (Das et al. 2010)

look grey or whitish gray or greenish (because of the presence of bile in them). Eventually by a slow process through lots of biochemical and genetic alterations those hepatic altered foci or hepatic nodules develop increasingly malignant cellular characteristics and are transformed into neoplasia without any further external stimulus or intervention (Farber & Sarma 1987).

4. Sequential changes in hepatocellular lineages leading to hepatocellular carcinoma

Etiology of hepatocellular carcinoma has probably been studied and analyzed in the best defined manner. Various groups of scientists have studied minutely and described the process of development of hepatocellular carcinoma during hepatocarcinogenesis. Other than initiation, promotion and progression models, Bannasch and his coworkers (Bannasch, 1995) have established and described the involvement of defined cellular lineages in the process of development of liver cancer. Predominant sequential cellular changes during the development of hepatocellular carcinoma commence with glycogenotic clear and acidophilic (due to proliferation of smooth endoplasmic reticulum) cell focal lesions and progress through intermediate phenotype of mixed cell population to glycogen poor basophilic (ribosome rich) cell phenotypes (Figure 4). The group has described few other cellular lineages. In the tigroid basophilic lineage, initially the cells have abundant highly ordered stacks of the rough endoplasmic reticulum and thereby they have uniqueness. The scientist group further reported that the lineage is common to the animals treated with a low dose treatment of hepatocarcinogen (Gournay et al., 2002). Another type of cellular lineage has been found to involve in the development of hepatocellular carcinoma. Rats when treated with non-genotoxic peroxisome proliferators or woodchucks chronically infected with woodchuck hepatitis virus showed foci with glycogen-poor cytoplasm containing abundantly granular acidophilic (mitochondria and peroxisome proliferators) and basophilic (ribosome) components (Bannasch et al., 1998). They named it amphiphilic cell lineage.

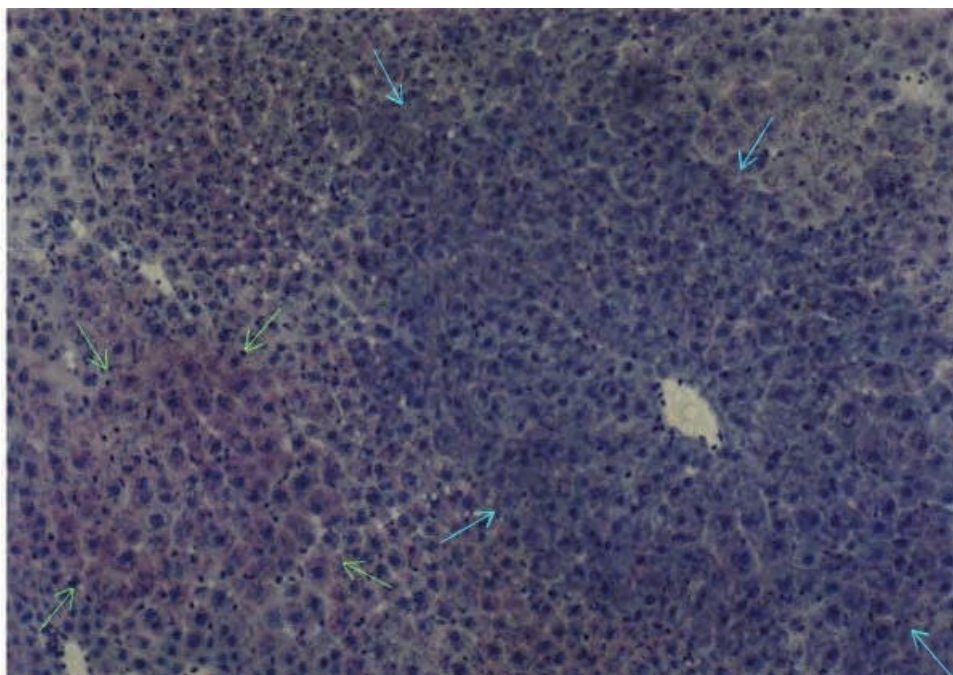


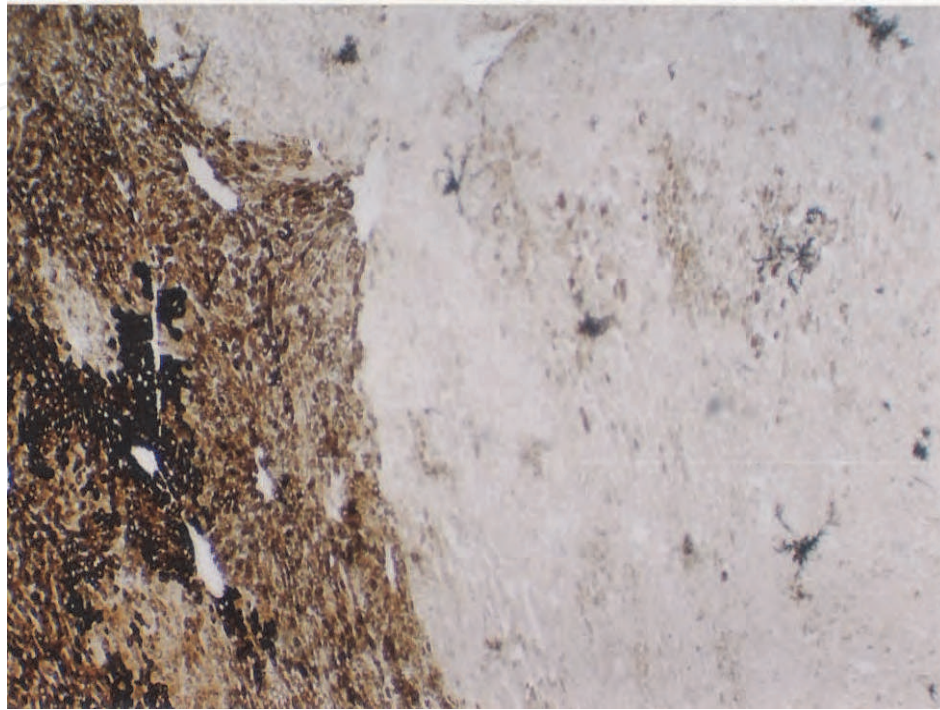
Fig. 4. Mixed cell focal lesion (shown by green arrows) and basophilic lesion (shown by blue arrows) in diethylnitrosamine-treated rat liver. (Mukherjee et al. 2007)

5. Biochemical modulation in hepatocellular carcinoma

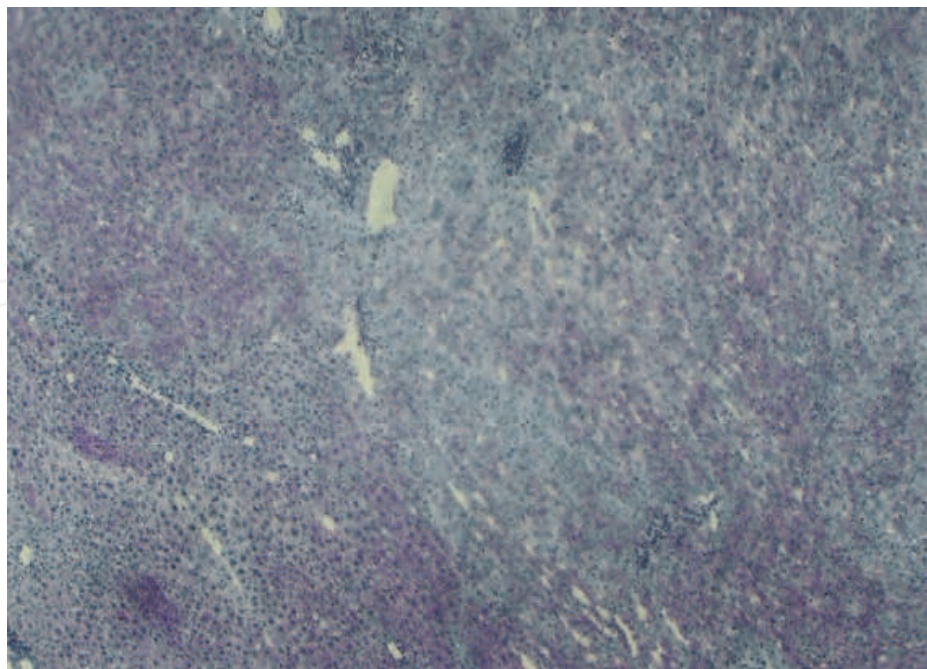
As discussed above, from the beginning of initiation process to the development of neoplasm, various biochemical and genetic changes occur in the affected cells. Some of these changes are well-distinguishable and vary along with the stages of development during various cancer processes including hepatocarcinogenesis. They have been described as preneoplastic or neoplastic markers, depending on the developmental stages (Pérez-Carreón et al., 2006). Liver is the largest organ in our body and it takes major role in metabolism. It has several enzymes which take part in metabolism and detoxification of various chemicals, including drugs. Most of these enzymes were discovered during investigation of drug metabolism in liver and thus they are called hepatic drug metabolizing enzymes. In liver, glutathione and related enzymes such as glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase and many other hepatic drug metabolizing enzymes and isoenzymes, such as glutathione-S-transferases (GSHT), UDP-glucuronyl transferases (UDPGT), cytochrome-c-reductase, cytochrome P-450 content and cytochrome b₅, have been identified as possible markers of preneoplastic and neoplastic hepatocytes (Mukherjee et al., 2005; Sarkar et al., 1994). They are categorized as Phase I and Phase II drug metabolizing enzymes/ isoenzymes. Variation in different such enzyme and isoenzyme levels, enzyme expression patterns assessed by histochemistry, lipid peroxidation profile, oxidative stress markers etc., during hepatocarcinogenesis, has been studied and reviewed by several workers (Sarkar et al., 1994). Conjugation of toxic metabolites with cellular macromolecules such as proteins and nucleic acids, may lead to several health problems including cancer. The enzymes (glutathione S-transferases, arylhydrocarbon hydroxylases, UDP-glucuronic transferases, cytochrome monooxygenases etc.) and isoenzymes (cytochrome b₅, cytochrome C etc.) are mostly involved in the detoxification process in liver. In Phase I hepatic metabolic reaction, oxygenation and hydroxylation reactions and in Phase II metabolic reaction in liver, glucuronidation and transferase reactions are very predominant. Glucuronic acid is transferred from uridine diphosphate glucuronic acid to a drug or phase I metabolite by the enzyme UDPGT (Vessey, 1996). Thus inefficient phase II processes cause increased deposition of phase I toxic metabolites. Another very important class of transferase enzymes is GSHT. They take enormous role in phase II detoxification process in liver. They detoxify electrophilic groups and thus inactivate even the function of carcinogens or mutagens. Satoh and Hatayama (Satoh & Hatayama, 2002) reported that specific different forms of GSHT are expressed during initiation, promotion and neoplastic cell populations. In the different developmental stages of hepatocellular carcinoma, glutathione peroxidase and reduced triphosphopyridine nucleotide (TPNH)-cytochrome-c-reductase activities, cytochrome b₅ and P-450 contents, glutathione content and superoxide dismutase and catalase activities were found to vary. Activities of these enzymes or their levels in hepatocellular carcinoma were always lower than those in initiation and promotion stages (Vessey, 1996). These reports suggest that the effects of Phase I hepatic drug metabolism is dwindled in hepatocellular carcinoma. The importance of glucose-6-phosphatase (G6P) in preneoplastic and neoplastic liver cannot be ruled out. The enzyme catalyzes in the final biochemical reactions of both gluconeogenesis and glycogenolysis (Nordlie & Sukalski, 1986; Shieh et al., 2003). This enzyme has an important role in blood glucose homeostasis (Nordlie & Sukalski, 1986). Histochemical demonstrations of G6P exhibited less pronounced activity in some cancer lesions and enhanced activity in the others. G6P-negative hepatocellular carcinoma (Figure 5) was also found to be basophilic (Mukherjee et al., 2007; Hwang et al., 2004).

Reports suggest that mutation in the G6P gene, G727T, leads to hepatocellular carcinoma (Nordlie & Sukalski, 1986).

Upon exposure of carcinogens, mutagens or other xenobiotics, reactive oxygen species are generated in the cells. Intracellular reactive oxygen species produce different types of DNA



A



B

Fig. 5. No predominant glucose-6-phosphatase expression in tumor area (A) of a basophilic tumor (B). (Mukherjee et al. 2007)

damage, including chromosomal aberrations, sister chromatid exchanges, and mutations (Dahm, 1996), leading to the initiation and/ or promotion and/ or progression of the cancerous process. Oxidative stress resulting from the imbalance of free radicals and the cellular antioxidant defense enzyme systems is reported to induce damage to cellular membranes and nuclear DNA, which results in lipid peroxidation and oxidative DNA damage, respectively (Dahm, 1996). Oxidation of the C8 of guanine which gives rise to the formation of modified base 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the most abundant types of oxidative DNA damage (Kasai & Nishimura, 1986). It is therefore considered as a sensitive biomarker for cancer development and an important molecular epidemiological assessment of cancer risk due to oxidative stress (Romano et al., 2000). The lipid peroxidation, another important chemical reaction owing to the oxidative stress during hepatocarcinogenesis, is known to influence tumor growth (Gelderblom, 2001).

6. Signaling of hepatocellular carcinoma: A special reference to the genes, Insulin-like-growth factor II (IGF II) and c-raf.1

Accumulation of mutations in a variety of genes transforms phenotypes of cancer cells. Mutations are found in several important genes, including p73, p53, rb, APC, DLC-1 (deleted in liver cancer), p16, PTEN, IGF-2, BRCA2, SOCS-1, Smad2 and Smad4, β -catenin, c-myc, and cyclinD1, in hepatocellular carcinoma (Farber & Sarma, 1987). These gene products normally modulate biochemical pathways that regulate cell death and cell proliferation. Deregulation of signaling pathways during the development of hepatocellular carcinoma affects normal cellular processes such as cell cycle and apoptosis. Many growth factors such as insulin-like growth factor I and II (IGF I/II) have ubiquitous role in the development of the disease. Raf/MEK/ ERK/MAP (Mitogen Activated Protein) kinase pathway, Akt pathway, Wnt pathway and Ink4A pathway are some of the predominant pathways involved in the neoplastic conversion of normal cells. However, here our focus is to establish the role of IGF II and c-raf.1 in Raf/MEK/ ERK/MAP kinase pathway during the development of hepatocellular carcinoma in hepatocarcinogenesis.

6.1 Insulin-like growth factor II (IGF II)

Insulin-like growth factor II (IGF II), a mitogenic polypeptide, has been widely implicated in the pathogenesis of neoplasm of different tissues including the liver of rats and men (Li et al., 1998; Mukherjee et al., 2005). This growth factor is found to express in neonatal life (in the first few days after birth) and then during neoplasia in rodents and men (Li et al., 1998; Mukherjee et al., 2007). IGF II in signaling for cancer cell proliferation is mediated through the Raf growth factor (Das et al., 2010). IGF II activates c-raf through signaling proteins such as Grb2 and Ras. Thus, the pathological implication of the overexpression of these two genes during the development of hepatocellular carcinoma cannot be ignored.

The correlation between IGF II expression and cancer development has been reported in a number of works (Mukherjee et al., 2005, 2007; Li et al., 1998). In a majority of liver carcinoma, IGF-II mRNA expression was reactivated and high levels of IGF-II expression were detected. We also investigated to understand the stage(s) at which IGF II gene activates during carcinogenesis. In our study, IGF II overexpression was observed in the early hepatic altered lesions (Figure 6A, B, C, D) and in hepatocellular carcinoma (Figure 7) (Mukherjee et

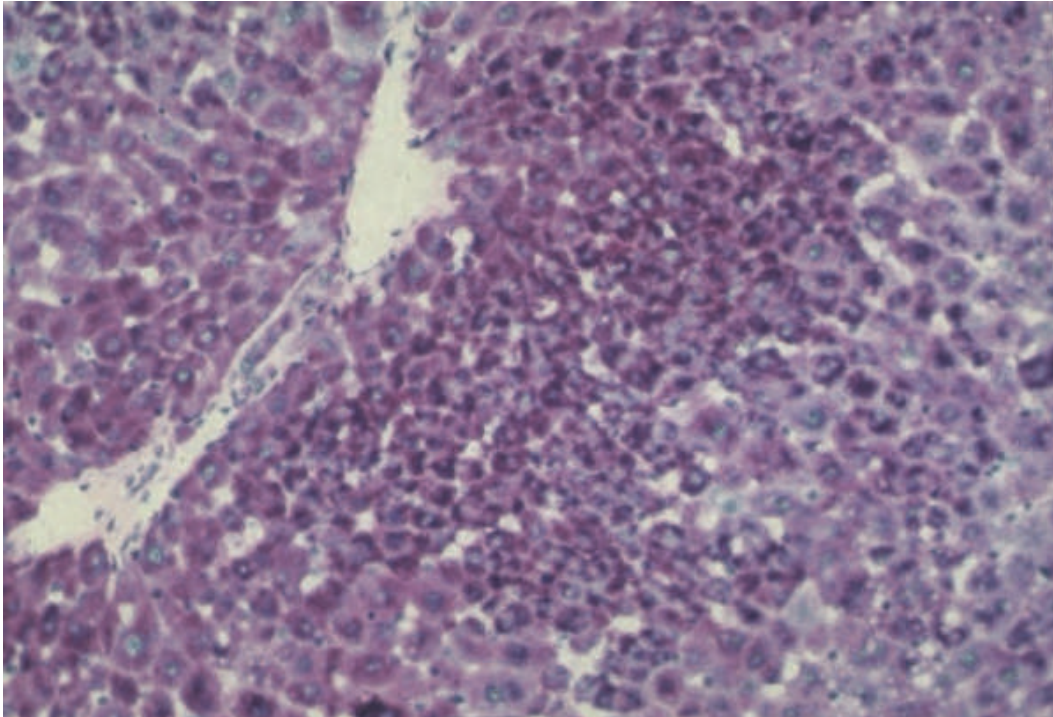


Fig. 6. A. Hepatic section of an experimental animal showing glycogen-stored early preneoplastic focal lesion with Periodic Acid Schiff reaction. (Mukherjee et al. 2007)

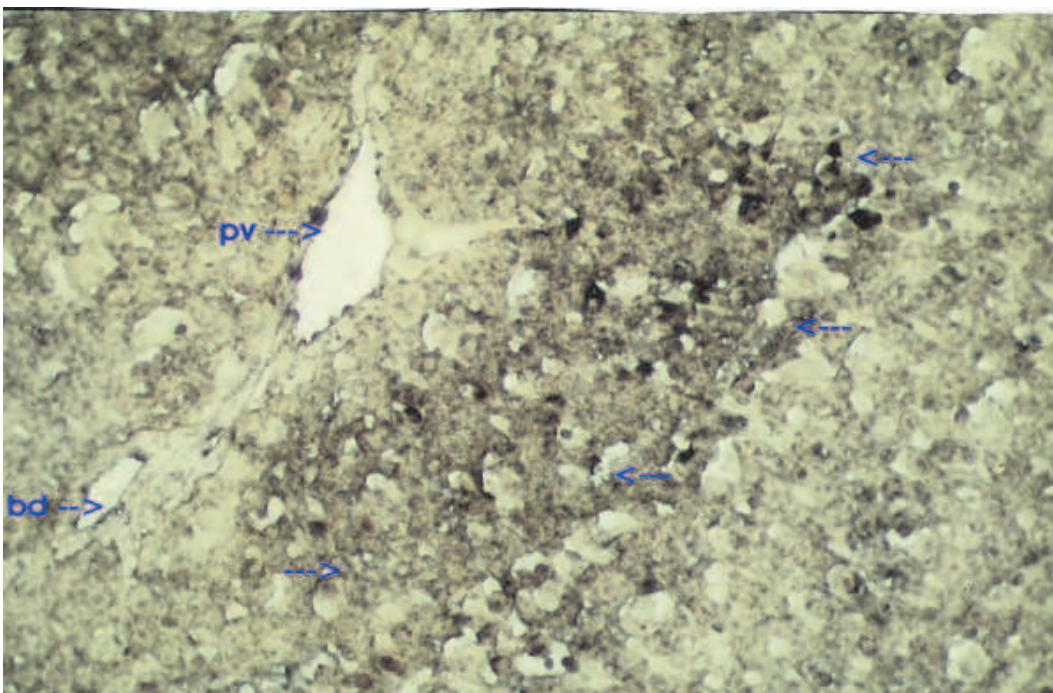


Fig. 6. B. IGF II mRNA-expressed glycogen storage early preneoplastic lesion detected with Digoxigenin-labeled antisense IGF II mRNA by *in situ* hybridization from the consecutive section. pv - portal vein. bd - bile duct. (Mukherjee et al. 2005)

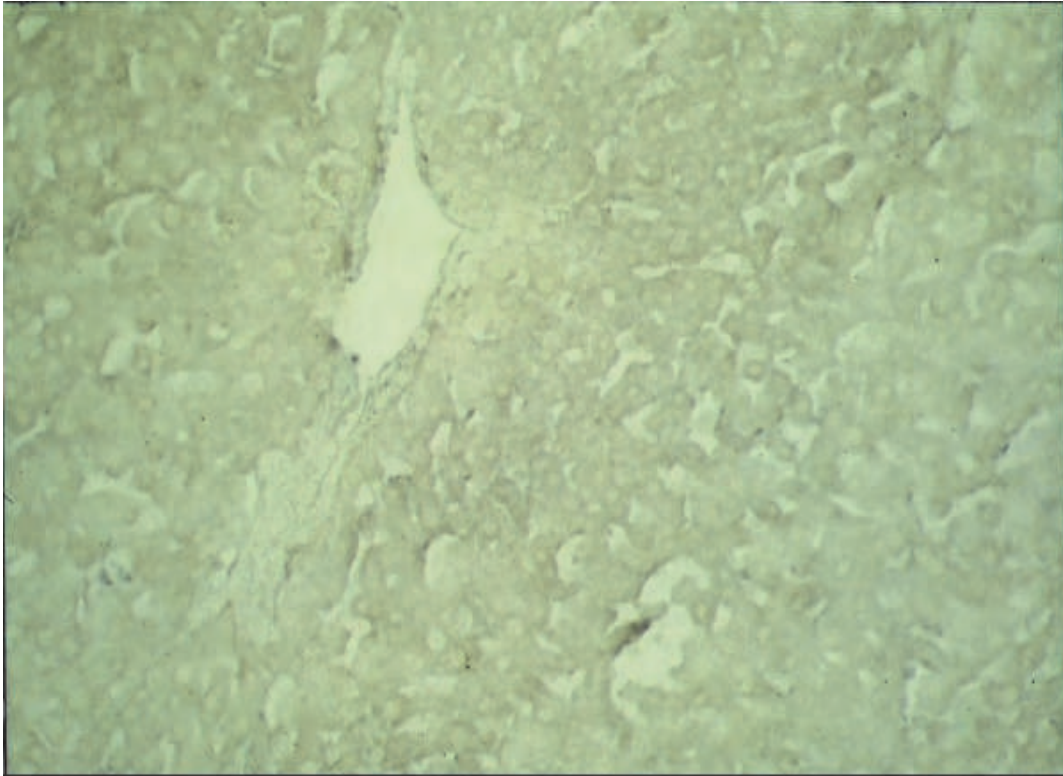


Fig. 6. C. Consecutive hepatic section after Digoxigenin-labeled sense IGF II mRNA treatment during *in situ* hybridization method. (Mukherjee et al. 2007)

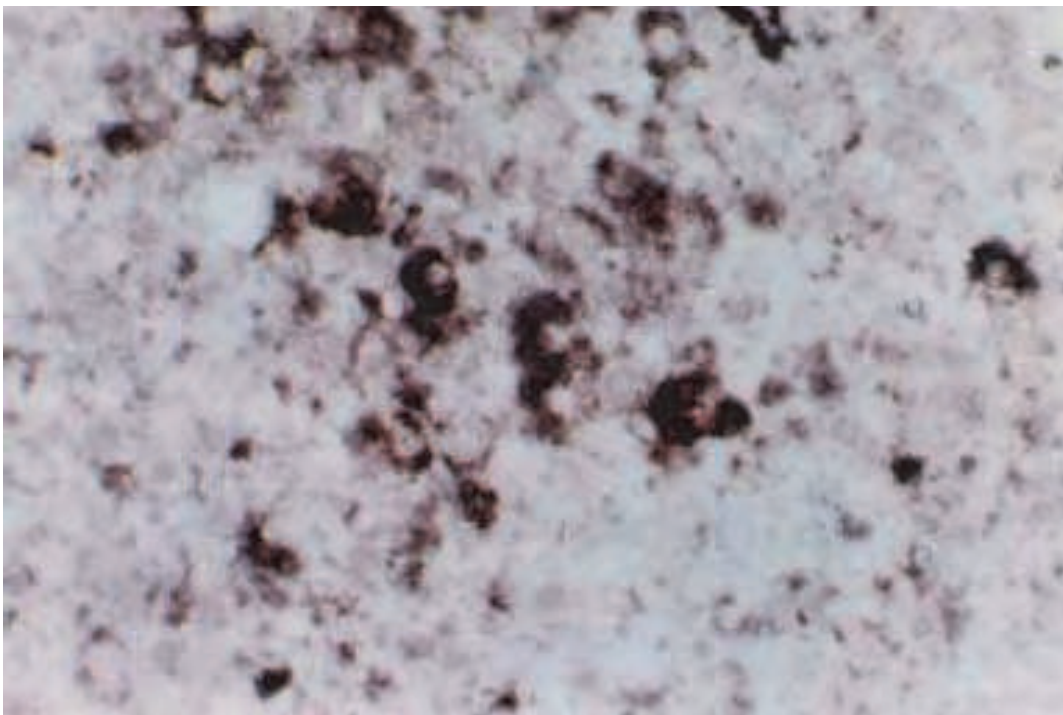


Fig. 6. D. IGF II-expressed one of the early preneoplastic lesions in an animal of initiation group. (Mukherjee et al.2005)

al., 2005). IGF II gene expressed in the sequence of events leading from glycogen-rich-acidophilic lesions to glycogen poor basophilic lesions through intermediate type lesions to hepatocellular carcinoma with an expression pattern of “high-low-high” in terms of degree of expression. More precisely, IGF II overexpression was found to be predominant in hepatocellular carcinoma and partially in early preneoplastic lesions. Thus, the gene has an essential role at the initiation stage of carcinogenesis (first few weeks) and during hepatocellular carcinoma development.

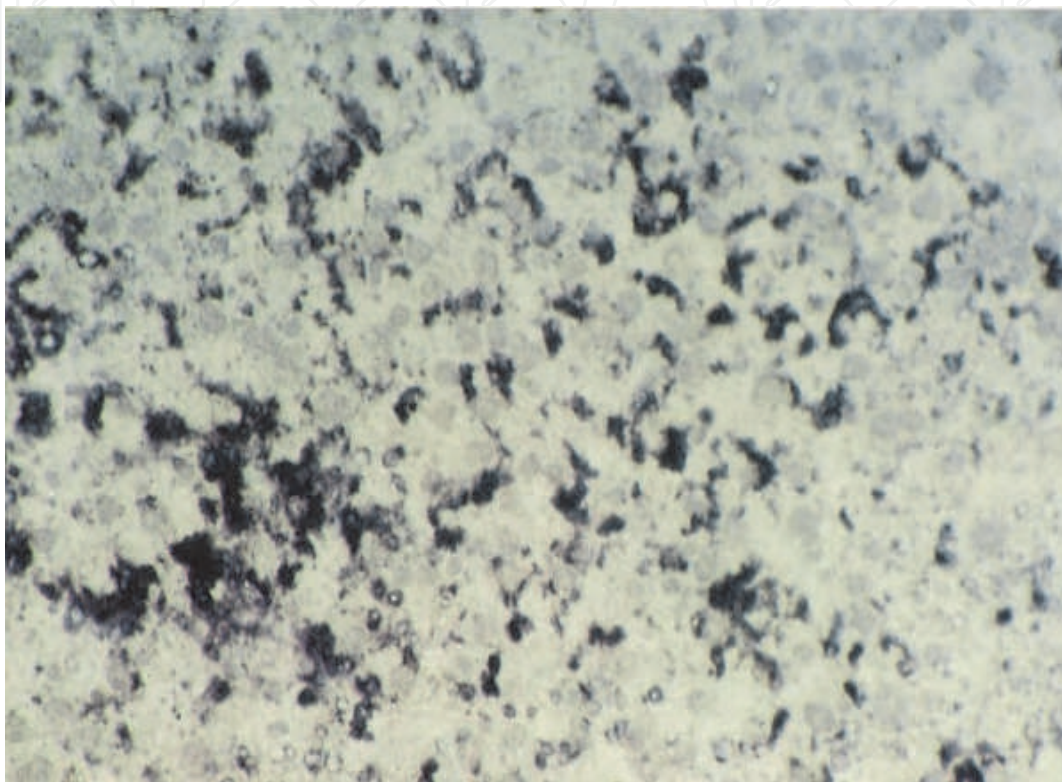


Fig. 7. IGF II mRNA expression in hepatocytes in tumor area (hepatocellular carcinoma) in experimental animals. (Mukherjee et al. 2005)

6.2 The Ras/raf/MEK/ ERK/MAP kinase pathway

The MAP kinase pathway has probably undergone the most extensive characterization in the process of development of hepatocellular carcinoma. Binding of a growth factor to a tyrosine kinase receptor causes receptor phosphorylation, leading to the formation of a molecular complex with an adaptor protein growth factor receptor bound-2 (Grb2), Grb2 associated binder 1 and signal relay protein SH-2 domain-containing tyrosine phosphatase-2. This is then localized in the plasma membrane. Other protein such as an exchange factor, Son-of-sevenless (SOS) joins. This complex activates ras while exchange GDP to GTP in the ras/raf/MEK/ERK/MAP kinase pathway. Ras/raf/MEK/ ERK/MAP kinase pathway is known to involve in cell proliferation, dedifferentiation, angiogenesis and cell survival process (Rapp, 1991). Activation of the components of this pathway has been reported to contribute to tumorigenesis, including liver cancer. The GTPase (Guanine nucleotides triphosphate)-Ras and the serine/threonine kinase raf (signaling regulators) regulate the signaling process immediately by activating raf which then phosphorylates the

mitogen/extracellular protein kinase kinases, MEK-1 and MEK-2. MEK proteins then phosphorylate the downstream extracellular signal-regulated kinase (ERK) signaling molecules, ERK-1 and ERK-2. GTPase-Ras is a switch protein which alternates between an active on state with a bound GTP and an inactive off state with a bound GDP (Polakis & McCormick, 1992). Activation of ERK-1 and ERK-2 regulates many target proteins and gene regulation proteins in cytoplasm and nucleus. Ras protein of this pathway is found to involve other signaling pathways such as phosphoinositol-3-kinase/Akt pathway, Phospholipase C/protein kinase C pathway and Ral guanine nucleotide dissociation stimulator pathway.

The c-raf.1 is a direct downstream effector of ras. The signaling molecule c-raf.1 is one of the three highly conserved members (raf A, raf B and c-raf.1) of the raf gene family, which code for serine threonine-specific protein kinases in ras-mediated signal transduction pathway (Daum et al., 1994; Rapp, 1991; Sebolt-Leopold, 2000). The c-raf.1 has a crucial role in diverse signal transduction pathways (Rapp, 1991). The c-raf.1 protein kinase has oncogenic potential and is found to be up-regulated in tumors (Störm SM et al. 1993; Stanton VP Jr et al. 1987) and highly expressed in hepatocellular carcinoma too (Rapp,1991; Störm et al., 1993). An excessive activation of the MAPK pathway was observed in hepatocellular carcinoma (Rapp,1991). This findings and another findings that shows overexpression of c-raf in hepatocellular carcinoma in all the 30 different tissue specimens as tested by Hwang et al. (Hwang et al., 2004) thus suggest the predominant role of the raf protein as well as ras/raf/MEK/ERK/MAP kinase pathway in hepatocellular carcinoma. In a phase-wise study (initiation/ promotion/ hepatocellular carcinoma) in rat hepatocarcinogenesis model (Mukherjee et al., 2007), we

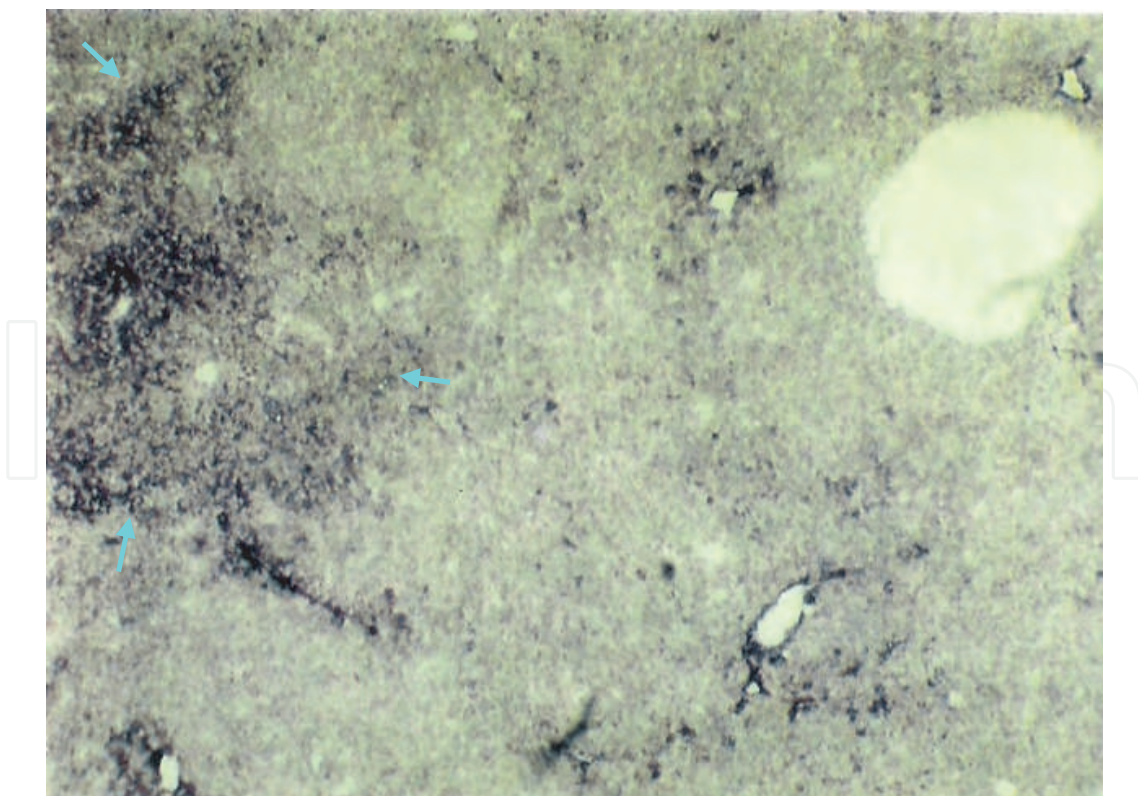


Fig. 8. A predominant raf-expressed late basophilic lesion (shown by arrows) in animal of cancer control group. (Das et al. 2009)

wanted to investigate the stage(s) at which c-raf.1 overexpression occurs. Our findings (as studied by in-situ hybridization) suggest that overexpression of the gene (Figure 8) occurs at the late stage basophilic focal lesions and in hepatocellular carcinoma. Further, c-raf.1 mediated activation of ras/raf/MEK/ERK/MAP kinase pathway may be a late stage phenomenon during the development of hepatocellular carcinoma and the activation may be either through c-raf.1 oncogenic mutation or by constitutive c-raf.1 activation by other deregulated proteins such as growth factors during hepatocarcinogenesis. Thus, constitutive activation of this pathway at one or more steps, particularly at ras or raf, can lead to a malignant state. However, no predominant difference is noticed between constitutive activation of Raf and Ras (Zang et al., 2002).

7. Conclusion

Unlike IGF II, c-raf.1 overexpression was observed in the late basophilic lesions associated with hepatocellular carcinoma. Thus, IGF II may have a role in activation of c-raf.1 signaling in the late stage of development of cancer. The role of raf.1 protein in IGF-induced signaling has been reported (Evert et al., 2004). But, when does it happen during the process of development of hepatocellular carcinoma? The c-raf.1 gene overexpression was predominantly found in hepatocellular carcinoma and late basophilic foci. Thus, the overexpression of c-raf.1 has been considered as a late-stage phenomenon during hepatocarcinogenesis (Bannasch, 2010). However, overexpression of c-raf.1 in very early lesion was also reported (He & Gascon- Barre, 1997). In our study, the dissection of animals after 7-8 weeks of carcinogenic insult did not show any raf.1-expressed lesions. Further, it was reported that c-raf.1 expressed in the basophilic tumors (Hwang et al., 2004). IGF II gene overexpression was noticed in the preneoplastic lesions and in hepatocellular carcinoma. On the other hand, overexpression of c-raf.1 gene was seen in the basophilic lesions associated with hepatocellular carcinoma as well as in tumor. While correlating the expression patterns of IGF II and c-raf.1, it suggests that IGF II-induced cellular signaling may be mediated through and/or affected by c-raf.1 in hepatocellular carcinoma and in the late stage of development of cancer. Thus, IGF II mediated c-raf.1 activation may drive late preneoplastic lesions towards neoplasia.

8. Acknowledgement

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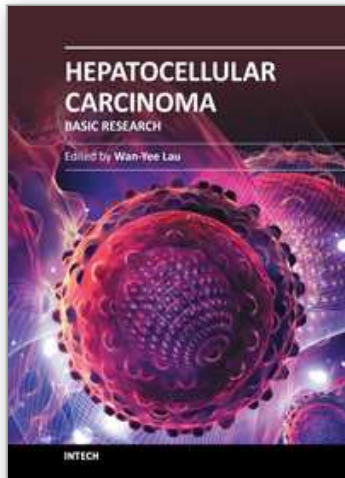
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Hepatocellular Carcinoma represents a leading cause of cancer death and a major health problem in developing countries where hepatitis B infection is prevalent. It has also become increasingly important with the increase in hepatitis C infection in developed countries. Knowledge of hepatocellular carcinoma has progressed rapidly. This book is a compendium of papers written by experts to present the most up-to-date knowledge on hepatocellular carcinoma. This book deals mainly with the basic research aspect of hepatocellular carcinoma. The book is divided into three sections: (I) Biomarkers / Therapeutic Target; (II) Carcinogenesis / Invasion / Metastasis; and (III) Detection / Prevention / Prevalence. There are 18 chapters in this book. This book is an important contribution to the basic research of hepatocellular carcinoma. The intended readers of this book are scientists and clinicians who are interested in research on hepatocellular carcinoma. Epidemiologists, pathologists, hospital administrators and drug manufacturers will also find this book useful.

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Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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