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Extended Abstract

Euro Biotechnology 2018: Elevated expression of cytosolic phospholipase A2 delta is associated with hepatocellular carcinoma progression: animal study validated with sera of liver cancer patients- Maryam Ranjpour Aghmiouni- Jamia Hamdard University

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Abstract

Hepatocellular carcinoma (HCC) is globally the fifth most common cancer with a high rate of morbidity and the third type of cancer causing maximum death among the patients diagnosed with cancers. Etiological influences such as hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, alcohol abuse, metabolic diseases and carcinogen exposure lead to chronic inflammation of liver and mutation causing heterogeneous HCC. Lack of clear symptoms, numerous relapse and inefficient therapy lead to poor prognosis and high mortality in patients diagnosed with HCC. Finding new generation noninvasive biomarkers to detect HCC at early stage would help reduce the rate of cancer-related mortality. Currently available markers, such as alpha-fetoprotein, do not have high sensitivity and search for novel markers is mandatory. Effective treatment and patient survival rate are dependent on the early diagnosis of HCC which can be provided based on the novel prognostic and diagnostic biomarkers.

In the present study, using animal model, we aimed to find out differentially expressed proteins that are associated with HCC initiation and progression to introduce as potential biomarker(s) or to target as therapeutic agent at very early stage of liver cancer initiation.

The experimental study involves analysis of rodent model in vivo which has previously been developed in our laboratory to study HCC. Further, the obtained data are validated with sera of clinically approved liver cancer patients.

Liver cancer induction and development of the rodent model

Liver cancer was chemically induced in 4-6 weeks old male Wistar rats weighing 80-100 g, by administrating chemical carcinogens DEN and 2-AAF as reported by our group earlier. Animal experimentation was performed following approval from Jamia Hamdard (New Delhi, India) Institutional Animal Ethics Committee formed for the Purpose of Control and Supervision of Experiments on Animals (project number 908). The protocol for HCC development in rats was essentially the same as previously described instruction. Briefly, the rats were kept in polypropylene cages while temperature was maintained at 25 ± 2°C with 12 hours cycle of light/dark in the animal house of Jamia Hamdard. These were fed ad libitum with free access to standard laboratory food (Amrut Laboratory, rat and mice feed, Navmaharashtra Chakan Oil Mills Ltd., India) and water daily. DEN (200 mg/kg body weight) and 2-AAF dissolved in 1% carboxymethyl cellulose (150 mg/kg body weight) were used as the initiator and promoter of HCC, respectively. Animals were randomly split up into two groups namely control and treated groups. Treated groups were further divided into two different groups namely, 1 M (sacrificed after one month) and 4 M (sacrificed after four months). The carcinogen treated animals were given a single high dose intraperitoneally (I.P.) of DEN, and after one week recovery period, the rats were administered with 2-AAF. Three doses of 2-AAF were orally administered on three alternative days among the first week of each month for entire study period (four months).

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Therefore, a total of 3 and 12 doses of 2-AAF were administered to the animals in the 1 M and 4M treated groups, respectively. The rats in control group received normal saline at the same schedule. The rats in the 1 M and 4 M groups were kept in a glass chamber containing cotton soaked with diethyl ether to be anesthetized and sacrificed at respectively one and four months after carcinogen treatment, respectively. At the time of sacrificing, the animals were perfused transcardially with saline and after their death they were dissected to excise livers for further analysis.

Neoplastic cell induction served as implication of cancer initiation in liver tissue of rats. Our method for animal model development is novel, as it neither requires carcinogen doses causing necrosis nor partial hepatectomy. The serum protein profile of carcinogen treated rats and controls were compared and differentially expressed proteins were identified. However, one of these proteins was further characterized as cytosolic phospholipase A2delta. Changes taking place in the expression of HCC- related proteins have been systematically monitored during various stages of HCC development, from the initiation of cancer to hepatotumorigenesis, when fully grown tumors were observed.

The importance of cytosolic phospholipase Aenzymes in cancer progression is of the considerable interests, as these enzymes play important role in the pathways associated with progression of cancer. This enzyme family controls cell proliferation, differentiation, survival and motility in almost all tissues. Their increased expression results in dysregulation and facilitates unlimited growth of tumors and metastasis for cancer cells. Significant role of these family members has been implicated in tumor progression and tumorigenesis. This pathway is activated in variety of cancers including HCC. Arachidonic acid, as a substrate for COXs and lipoxygenases, is a necessary factor that producing bioactive eicosanoids and platelet activating factor which, in turn, regulate inflammation, tumor cell proliferation and motility, differentiation, survival, invasion, angiogenesis and metastasis in HCC.

We observed elevated levels of cytosolic phospholipase A2 delta in the serum of HCC rats and in human patients with liver cancer. This suggests that is one of the important factors associated with HCC initiation and progression leading to hepatotumorigenesis. Elevation of cytosolic phospholipase A2 delta expression in liver cancer might be associated with dysregulation of lipid metabolism and liver damage, causing cancer initiation in tissue at precancerous stage, while the epithelial cells are actively proliferating.

Taken together, the present study suggests that evaluation of cytosolic phospholipase A2 delta concentration, alone or in consolidation with other conventional markers, may provide critical knowledge for the early noninvasive disclosure of HCC. Moreover, cytosolic phospholipase A2 delta might also be served as a potential target to find out the status and progression of liver cancer.

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