Archives of Biological and Biomedical Research
An International Journal

Expression of C-KIT Tyrosine Kinase Receptor, Epidermal Growth Factor, and p53 Proteins in the epithelial and mesenchymal cells of Hepatoblastoma: Preliminary Findings

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Abstract:

Background: Overexpression of receptor-type protein tyrosine kinases (PTKs) and alterations of p53 are well known events in neoplastic cells and are associated with an aggressive tumor phenotype. To date, our knowledge about PTKs and p53 expression in the neoplastic cells (epithelial and mesenchymal cells) of hepatoblastoma is incomplete**. Objectives:** This study tries to address this issue and test the hypothesis that "PTKs receptors and p53 protein expression is altered in hepatoblastoma". **Materials and methods:** The expression patterns of PTKs (epidermal growth factor receptor and KIT) and tumor suppressor p53 protein) was examined in a case of hepatoblastoma using specific antibodies and immunoperoxidase staining methods. **Results:** Cytoplasmic PTKs and p53 protein expressions were seen in both glandular and mesenchymal component of the hepatoblastoma. Some nuclear p53 protein expression was also seen. **Conclusions:** The expression of these proteins in hepatoblastoma suggests their possible roles in histogenesis of these lesions.

Keywords: C-Kit, EGFR, p53 , hepatoblastoma

1 Introduction:

Hepatoblastoma is a common primary liver tumor in children. It represents 50% of liver malignancies in children; and up to 0.5% of all pediatric tumors. It has been proposed that hepatoblastoma is derived from either mesoderm or endoderm. Others suggested an origin from a single pluripotential stem cell or from a single uncommitted liver precursor cell with divergent differentiation. The genetic changes in hepatoblastoma include trisomy 2 and abnormalities of chromosome 11 and 17. Histologically, hepatoblastoma is composed of a varied mixture of both epithelial and mesenchymal cell types. Teratoid features are seen in 34% of hepatoblastoma and include presence of keratinized squamous epithelium, intestinal epithelial, skeletal muscle, mature bone and cartilage, melanin and neuroectodermal structures. The existence of teratoid features suggests the presence of stem cells in hepatoblastomas [1].

Receptor protein tyrosine kinases (PTKs) are central molecules in cellular signaling processes that regulate cell growth and differentiation by catalyzing protein

phosphorylation and dephosphorylation. Several PTKs such as EGFR (Epidermal Growth Factor receptor) and c-Kit regulate tumor growth. Their targeting by specific PTKs inhibitors represents a novel therapeutic method for treating cancers. Different molecules targeting PTKs are either under clinical investigation or are already approved [2]. The proto-oncogene c-Kit is the cellular homologue of v-kit, an oncogene derived from the feline retrovirus HZ4-FeSV. c-Kit (now known as KIT) encodes a 14.5-kD transmembrane receptor (KIT or CD117) belonging to class III receptor tyrosine kinase family. KIT operates in cell signal transduction in several cell types [3]. Under normal condition, KIT is activated (phosphorylated) by binding of its ligand, the stem cell factor. This leads to a phosphorylation cascade activating several transcription factors that control apoptosis, cell differentiation and proliferation. Pathologic activation of KIT through gain-of-function mutations leads to neoplasia of KIT-dependent and KIT-positive cell types [1]. Epidermal growth factor receptor (EGFR) is a protooncogene that induces tyrosine kinase activity. It is characterized by an extracellular ligand-binding domain, an internal kinase domain, and a carboxyl-terminal domain that contains multiple tyrosine residues. Upon binding of EGF, the receptor dimerizes and becomes phosphorylated, which in turn act as docking sites for multiple signaling proteins that contain SH2 domains [2].

p53 is a stress response gene that encodes a 53 KDa oncosuppressive nuclear protein with a Mr of 53,000. This protein can activate twenty different promoters, repress twenty-six different promoters and enhancers, and can interact with more than thirty-five cellular and viral proteins. Also, p53 is a synaptic point where upstream and downstream cross talks dictate the final destiny of the cell. The components of the p53 upstream pathway have not been identified and seem most likely to include protein kinases that phosphorylate p53 protein. The downstream molecules include several p53 "effector" genes [4]. To date, our knowledge about the expression pattern of PTKs and downstream p53 protein in hapatoblastoma is incomplete. To address this issue, and test the hypothesis that "PTKs receptors and p53 protein expression is altered in hepatoblastoma"; a case of hepatoblastoma was evaluated for the expression of these proteins using immunoperoxidase staining methods and specific antibodies.

2 Materials and Methods:

Tissue specimen: Formalin- fixed, paraffin embedded tissue specimens representing a case of hepatoblastoma was used. The materials belong to a 24-month-old boy who presented with history of abdominal swelling of a gradual onset and a rapidly progressive course. His history was unremarkable and did not contain previous complaint of ill health. Abdominal examination revealed a swelling in the right hypochonderium and epigastric region. Computed tomography and magnetic resonance imaging studies revealed a huge liver mass (10 cm). The tumor showed high vascularity and marked enhancement on computerized tomography. Extensive investigations did not identify tumors elsewhere. A computed tomography guided biopsy was obtained, submitted for pathological evaluation and diagnosis of hepatoblastoma was established.

Immunohistochemistry:
Immunohistochemical analysis was carried out as previously described [5]. Sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked and sections were subjected to heat-induced antigen retrieval. Non-specific protein binding was blocked. Sections were incubated with primary antibodies for 30 min at room temperature (Clones 104D2, DO-7, H11for C-KIT, p53 and EGFR, respectively, DAKO Corp., California, USA). A catalyzed signal amplification system (DAKO Corp., California, USA) was used according to manufacturer instructions.

Positive controls: Positive controls consisted of mastoctyoma (KIT), adenocarcinoma

(EGFR) and squmaous cell carcinoma (p53). Reactivity was identified as brownish cytoplasmic and membranous staining (KIT), cytoplasmic (p53, EGFR) and nuclear relativities (p53).

Negative controls: Additional sections, running in parallel but with omission of primary antibody served as negative controls.

3 Results:

All specimens were batch-stained in same run and the experiments were repeated three times [5]. The positive and negative controls were consistently positive and negative respectively indicating the validity of the staining results.

Immunostaining revealed a strong cytoplasmic expression of KIT, EGFR and p53 proteins. Some neoplastic cells (~20%) show nuclear p53 protein expression. A summary of these results is shown in **Figure 1**.

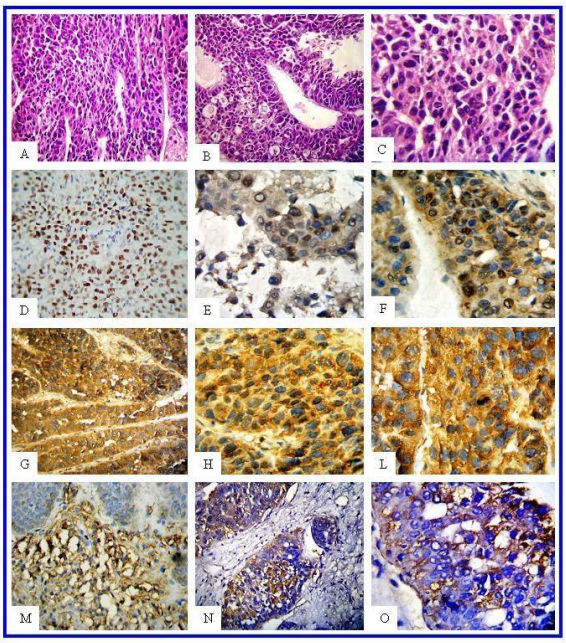

Figure 1: Immunostaining of p53, epidermal growth factor and KIT proteins in hepatoblastoma. The tumor is composed of a varied epithelial and mesenchymal components (A). The epithelial component consists of glandular structures (B) and individual mucin secreting cells (arrowhead). The mesenchymal element consists of primitive round, oval and short spindle shaped cells (C). Positive nuclear and cytoplasmic staining for p53 in the control (D, squamous cell carcinoma) and hepatoblastoma (E-F). A prominent reactivity was seen for epidermal growth factor in the control tissue (G, control adenocarcinoma) and hepatoblastoma (H-L). A positive cytoplasmic and membranous staining for KIT proteins is seen in the control (M, mastocytoma) and the neoplastic cells (N-O) seen both in the epithelial (D and E) and mesenchymal cells (D and F).

4 Discussion:

The description of PTKs receptors and some downstream nuclear proteins is critical for understating of histogenesis of different tumors. Also, it is of paramount importance for future implication in prognostication and therapy. Nevertheless, our knowledge about the expression pattern of these proteins in hepatoblastoma is incomplete. The present study was performed to elucidate this issue. It revealed an intense cellular localization of these proteins. This prominent expression suggests possible roles for these molecules in the histogenesis of hepatoblastoma. Possible mechanisms by which these PTKs receptor and nuclear proteins can share to histogenesis include their ability to promote angiogenesis and inhibit endothelial apoptosis.[7 ,6]

The prominent expression of KIT protein in both mesenchymal and epithelial cells indicates pathologic activation of KIT tyrosine kinase receptor in hepatoblastomas. KIT protein expression also suggests a single origin for both epithelial and mesenchymal elements in these lesions and raises the notion that hepatoblastoma arises from a pluripotential cell. The latter can differentiate into both epithelial and mesenchymal elements. The strong expression of PTKs receptor proteins (KIT and EGFR) in the lesional cells may be explained by presence of PTKs receptor ligands in these cells together with a receptor-mediated internalization of PTKs receptor proteins. These cells may have membrane-type PTKs ligands, and therefore can stimulate PTKs receptor expression 11. It is still possible that PTKs receptors and their ligands help provide a unique milieu for biological behavior of the hepatocytes [7]. Pathologic activation of PTKs receptors through gain-of- function mutations leads to neoplasia of PTKs receptor -positive cell types such as gastrointestinal stromal tumors (KIT)

and breast carcinomas (EGFR) [1]. Therefore, altered expression of PTKs receptors may have a role in development of tumors of the liver. Several studies indicated that PTKs receptor ligands such as stem cell factor (KIT ligand) plays a crucial role in the development, migration, survival, proliferation and neoplastic transformation of several cell types [1, 8]. Interestingly, pharmacologic manipulations of KIT and EGFR signaling pathways are useful for treatment of some aggressive neoplasms. Anti-CD117 and EGFR (STI571 and ZD1839) are used as adjuvant therapy in melanomas and carcinomas [9].

The immunoreactivity for p53 protein in this study concurs with previous reports [10, 11]. p53 cytoplasmic staining in hepatoblastoma is in agreement with findings in several cancers (colorectal and lung cancers) and may merely reflects cell cycle fluctuations of p53 protein at the checkpoints, not underlying p53 gene defects. The evidently low nuclear p53 staining in hepatoblastoma is suggestive of an intact nucler localization signal that is rarely, if ever, the site of ongoing p53 mutations. As the presence of an intact nuclear localization signal would allow the mutant 53 protein to stay in the nucleus and exert its dominant negative function, it is conceivable that these subsets of cells would have more unfavorable outcome. The clinical and prognostic values of p53 staining localizations in hepatoblastoma remain unclear [4].

To conclude, this study reports the expression pattern of PTKs and some nuclear proteins in hepatoblastoma. It supports the thesis that stem cells (KIT positive) play a role in the histogenesis of hepatoblastoma. The findings reported here have two potential ramifications. First, PTKs and nuclear protein expression pattern may help establish a foundation for future analyses of these proteins in other blastomas. Second, inhibitors of these proteins may be used as an adjuvant therapy for these blastomas. However, further studies on a large series of blastomas is mandatory to substantiate these implications.

Table 1: The antibodies used in the immunohistochemical evaluation

Antibody	Control	Retrieval	dilution	Incubation time	Distribution of staining
KIT	Mastocytoma	Citrate,pH=6	1:100	30 min at 37 °C	Cytoplasmic, membranous
p53	Squmaous cell carcinoma	Citrate,pH=6	1:100	30 min at 37 °C	Cytoplasmic and nuclear
EGFR	Adenocarcinoma	Citrate,pH=6	1:200	30 min at 37 °C	Cytoplasmic

Table 2: Staining patterns of KIT, p53 and EGFR proteins in hepatoblastoma

Antibody	Intensity and localization of staining	
	Epithelial element	**Mesenchymal element**
KIT	+++ (cytoplasmic)	+++ (cytoplasmic)
p53	+++ (membranous and cytoplasmic)	+++ (membranous and cytoplasmic)
EGFR	++ (cytoplasmic)	++ (cytoplasmic)

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