BASIC–LIVER, PANCREAS, AND BILIARY TRACT

Diverse Expression of ErbB Receptor Proteins During Rat Liver Development and Regeneration

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Background & Aims: **The protein expression and interactions of the ErbB receptors were examined in different liver proliferation models in vivo and in vitro, including ontogeny and regeneration following partial hepatectomy.** ***Methods:*** **Expression and tyrosine phosphorylation status of specific ErbB proteins were studied by immunologic methods.** ***Results:*** **The epidermal growth factor receptor, ErbB2, and ErbB3 were the only ErbB proteins detected in the liver parenchyma on embryonic day 19. ErbB2 disappeared by the third week after birth and could not be appreciably induced in the adult animal by partial hepatectomy. ErbB2 was also detected in multipotent stem (RLE) and hepatoma (H4IIe) cell lines as well as in fetal, but not adult, hepatocyte cultures. Only epidermal growth factor receptor and ErbB3 were detected in adult liver, and both showed circadian variation in protein expression. ErbB4 was not detected in any model. Patterns of ligand-induced ErbB phosphorylation differed between fetal and adult hepatocytes.** ***Conclusions:*** **Complex and independent programs regulate the ErbB receptors, with implications for differential cell signaling in hepatic development and regeneration.**

The ErbB proteins (ErbB1 to ErbB4) are transmembrane tyrosine kinase receptors that were named for their homology to the avian erythroblastosis virus oncogene *v-erbB* and are often referred to as HER1 to HER4 in human tissues.[1] ErbB proteins are widely expressed in human and animal tissues, notably in cells of epithelial or neuroectodermal origin. They have a molecular mass between 170 and 185 kilodaltons and have significant sequence identity with each other, particularly in the tyrosine kinase domain.[1] When activated by ligands, they generate diverse cellular responses that include proliferation, growth arrest, differentiation, and transformation.[2] The prototype in this family is the epidermal growth factor receptor (EGF-R or ErbB1). Its tyrosine kinase domain is activated by binding of peptides of the epidermal growth factor (EGF) family to the extracellular, ligand-binding domain. Hepatocytes of the mature liver express the highest levels of EGF-R of any nontransformed cell, suggesting an important role for ErbB signaling in normal liver function.[3]

Ligands for ErbB receptors comprise a large and expanding family of more than 20 EGF domain-containing proteins encoded on at least 9 genes. These ligands are initially synthesized by many tissues, including the liver, as membrane-anchored precursors that can be cleaved into mature soluble growth or differentiation factors by multiple metalloproteinases, including tumor necrosis factor α converting enzyme (TACE or ADAM17).[4] Three families of ErbB ligands are defined on the basis of ligand interactions with distinct subsets of ErbB receptors. The ligands transforming growth factor α, EGF, and amphiregulin bind exclusively to the EGF-R, whereas a second family, the heregulins or neuregulins, binds exclusively to ErbB3 or ErbB4. A third family, comprised of betacellulin, heparin-binding EGF-like growth factor, and epiregulin, binds to both EGF-R and ErbB4. ErbB2 (or neu), despite the presence of an extracellular ligand binding domain, does not bind any of the known EGF homologues or heregulins and may not have a ligand.

In response to ligand binding, ErbB proteins form either homodimers or heterodimers, depending on the available repertoire of family members in a given cell.[5] Receptor dimerization leads to activation of the ErbB tyrosine kinase domains by transphosphorylation, which in turn results in signal propagation. The sole exception to the requirement for ligand activation of ErbB signal-

Abbreviations used in this paper: EGF, epidermal growth factor; EGF-R, epidermal growth factor receptor; PH, partial hepatectomy; SH, sham hepatectomy.

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ing may be cells that overexpress ErbB2, in which mass effect seems to induce homodimerization and signaling in the absence of an ErbB2 ligand. Individual ErbB molecules favor certain family partners over others (e.g., ErbB2, when present, is always the preferred pairing partner for any other ErbB protein[6]), and individual EGF ligands may preferentially induce specific ErbB combinations as well. Because each ErbB family member contributes unique intracellular docking sites for recruiting and activating signaling molecules, different ligands can propagate different downstream signals and cellular outcomes depending on the receptors available for dimerization.[7] Thus, a multitude of unique signaling pathways can be activated depending on the ligands and receptors available to any given cell.

ErbB proteins play an important role in mammalian development and cancer. Gene disruption of ErbB proteins or their ligands has resulted in a number of embryologic or neonatal phenotypes associated with severe cardiac, neural, or intestinal abnormalities,[8] although no liver phenotype has yet been described. There is a well-developed literature on the involvement of both EGF and transforming growth factor α in both carcinogenesis and regeneration of liver. Increased expression of normal or mutated ErbB proteins results in a poor prognosis for patients with breast cancer or other solid tumors.[9] In the liver, the incidence and expression of the ErbB2/neu oncoprotein are increased in patients with hepatitis C with chronic active hepatitis or hepatocellular carcinoma compared with asymptomatic carriers.[10–15] Indeed, transfection of the *c-ErbB2/neu* gene into human hepatocarcinoma cells increased their metastatic potential.[16] Such findings make the ErbB molecules and their signal transduction pathways compelling targets for cancer therapy.[17]

We have reported that the only ErbB proteins expressed in the adult rat liver are EGF-R and ErbB3.[18,19] Because ErbB proteins play a striking role in the development of other tissues and because the adult rat liver lacks both ErbB4 and the almost ubiquitously expressed ErbB2, we examined the ontogeny of ErbB expression in the liver. Our studies show a developmentally and circadian regulated pattern of ErbB expression in the liver. These patterns provide a basis for differential combinatorial ErbB signaling during development and at different times of day. Although ErbB2 is not expressed in the normal or regenerating adult liver or in primary hepatocytes derived from adult animals, we now report strong ErbB2 expression in the fetal and neonatal liver, in stem-like RLE 13 cells, and in the H4IIe hepatoma cell line.

Materials and Methods

Animals

Male Sprague–Dawley rats (150–200 g) from Harlan (Indianapolis, IN) were housed under conditions of regulated lighting (lights on from 6 AM to 6 PM) and ad libitum access to water and Purina rodent chow (Ralston-Purina, St. Louis, MO). For the developmental study, timed pregnant female rats were obtained from Harlan and rat pups harvested surgically or postpartum. All protocols used were approved in advance by the Animal Use Subcommittee of the Vanderbilt Animal Care Committee.

Partial hepatectomy (PH) was performed with the rats under ether anesthesia and involved the ligation and resection of the left lateral and median hepatic lobes as described.[20] Sham-operated controls (SH) were similarly anesthetized and their livers brought outside the peritoneal cavity but not ligated or excised. Because of the diurnal variation in liver DNA synthesis after PH, in one study,[21] all rats underwent surgery between 8 AM and 12 PM. In a follow-up study, all animals were killed during this period and underwent surgery at different circadian times.

Primary Cell Cultures

Fetal hepatocytes (E-19) were isolated according to the protocol of Curran et al.[22] Briefly, the female was anesthetized and the pups were delivered by cesarean section. Each pup was decapitated and its liver was removed and placed in a Petri dish containing KRH buffer (Krebs-Ringer solution with 20 mmol/L HEPES, pH 7.4). After the livers were harvested, they were finely minced and then transferred to a 50-mL conical tube. The liver bits were allowed to settle, and the medium was aspirated and replaced with 20 mL KRH plus 5 mmol/L ethylenediaminetetraacetic acid. After a 5-minute incubation (with rocking) at 37°C, the liver bits were again allowed to settle and the medium was aspirated and replaced with 20 mL collagenase solution (containing 0.05% collagenase and 0.1 mg/mL deoxyribonuclease 1) and allowed to incubate while rocking at 37°C for 10 minutes. The bits were then gently dispersed by drawing them through a 5-mL pipette and returned for an additional 10 minutes of incubation. After a final dispersal, the entire volume was filtered through 80-μm Nytex mesh and centrifuged at 20*g* for 1 minute. The pellet was gently resuspended in Williams' Medium E, centrifuged a final time, and suspended in Williams' Medium E plus 5% fetal bovine serum. Cell count and viability were ascertained by trypan blue, and viable cells were plated at a concentration of 1.5 million per dish and allowed to attach overnight at 5% CO_2 and 37°C. The cultures were allowed to grow for 4.5 days and had 2 additional medium changes before experimental media were added. Adult hepatocytes were obtained as previously described,[19] and experiments were performed after 12 hours of culture to minimize a down-regulation of ErbB3 that occurs in cell culture.