Novel cell line and animal model for human biliary tract cancer

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Abstract

Biliary tract cancer still has a poor prognosis with approximately 8,000 cases reported annually in USA. The most promising therapy for the patients with BTC is surgical resection. Chemotherapy is also significant to improve the prognosis, but the available chemotherapeutic agents and anti-cancer effects are limited so far. Thus, novel anti-cancer drugs and regimens are urgent issue. We established a novel cell line from human intraductal papillary neoplasm of the bile duct, which is a novel biliary tract malignant entity. We also developed an orthotopic engraft model by inoculating human gallbladder cancer cells into nude mice. In this review, we summarized characteristics of a novel cell line and an animal model of human BTC.

Introduction.

Biliary tract cancer (BTC) still has a poor prognosis with approximately 8,000 cases reported annually in USA and is the second most common after hepatocellular cancer among primary liver cancers. The most promising therapy for the patients with BTC is surgical resection. While only about 70% of the patients are able to undergo surgical resection, many of them suffer from recurrent disease even after resection [1, 2]. Chemotherapy is also significant to improve the prognosis, but the available chemotherapeutic agents and anti-cancer effects are limited so far [3, 4]. Thus, novel anti-cancer drugs and regimens are urgent issue. The scarcity of cell lines from human BTC and orthotopic engraft models of BTC make it difficult to advance the basic research [5-11]. We have recently established more than 20 cell lines from human pancreatic cancer and described the characteristics of these cell lines [12]. Subsequently, we established a novel cell line from human intraductal papillary neoplasm of the bile duct (IPNB), which is a novel biliary tract malignant entity that is classified as premalignant or early stage bile duct cancer [7]. We also developed an orthotopic engraft model by inoculating human gallbladder cancer cells into nude mice [2]. In this review, we summarized characteristics of a novel cell line and an animal model of human BTC.

Establishment of a new cell line from human intraductal papillary neoplasm of the bile duct (IPNB).

IPNB was identified as a novel disease entity distinct from conventional cholangiocarcinoma [13]. In general, IPNB is characterized by rich mucin-production and relatively slowly growing tumor. IPNB is regarded as a conceptual hepatobiliary counterpart of intraductal papillary mucinous neoplasm of the pancreas (IPMN). But the detailed characteristics of IPNB remain uncertain. We reported that CD133, pan-stem cell marker, was expressed in conventional intrahepatic cholangiocarcinoma and hilar cholangiocarcinoma, but not in

IPNB [14]. CD133 expression is also lacking in IPMN, supporting the hypothesis that IPNB is the hepatobiliary counterpart of IPMN [15].

We have tried to establish new cell lines from resected BTC tumor tissue. Then, we successfully developed single cell line from human IPNB (KBDC-11) [7]. To our knowledge, this is the first cell line from human IPNB. Available other bile duct cancer cell lines are established from advanced cancers, and these can be mainly used to the validation of the efficacy of anti-cancer drugs or molecular-targeting drugs as well as the search for predicting molecular biomarkers [16-18]. In humans, cholangiocarcinoma is thought to develop from precursor lesions, including biliary intraepithelial neoplasias (BilIN) or IPNB. Therefore, our novel cell line from IPNB can provide the advantage in the study for molecular characteristics of bile duct carcinogenesis. Wang et al. reported that CD133 expression is related to tumor invasiveness or increase of the sensitivity to radiation or gemcitabine in bile duct cancers [18]. However, KBDC-11 was lack of CD133 expression. We thus need to clarify how CD133 functions in carcinogenesis or tumor phenotype.

To date, only a few BTC cell lines are available, since the success rate of establishing BTC cell line is extremely low, commonly less than 1% [18]. Although we have successfully developed many pancreatic cell lines [12], the success rate of establishing BTC cell line was only 2.1% (1/48) in our lab (unpublished data). One possibility is to assume that bile has detergent properties and often contains bacteria.

Animal model of biliary tract cancer.

Kiguchi et al. have generated a transgenic mouse, which overexpresses erbB2 under the control of the bovine keratin-5 promoter and have extremely high incidence of gallbladder cancer [19]. Kiguchi et al. and we utilized this model to test the efficacy of anti-cancer drugs or inhibitors for biliary tract cancer [19, 20]. On the other hand, mutations in isocitrate dehydrogenase (IDH) are the most common genetic alterations in human intrahepatic cholangiocarcinoma (IHCC).

Saha et al. have generated a mutant IDH-2 and Kras transgenic mouse. This model developed IHCC between 33 to 58 weeks. They also revealed that mutant IHD blocks hepatocyte differentiation via suppression of HNF-4α [21]. Their model for IHCC pathogenesis is that mutant IHD and another extra hits (e.g. Kras mutation) cooperatively incite oval cell expansion, increasing grades of BilIN, and development of IHCC [21]. However, these genetically modified animals can not recapitulate cancer phenotype, such as tumor microenvironment or metastasis.

In place of use of transgenic mice, we have developed simple methods for inducing orthotopic gallbladder cancer xenografts in the nude mice [2, 22]. The orthotopic xenografts is assumed to have advantages compared to ectopic (subcutaneous) xenografts in several ways, such as tumor microenvironment and reproducibility of distant metastasis [23]. However, since the biliary tract is luminal organ with thin walls, it is difficult to induce orthotopic engraftment with BTC. We utilized NOZ cells, isolated from human gallbladder cancer. NOZ cells are characterized by high frequency of tumor formation and metastasis. Briefly, mouse cystic duct was ligated and bile juice in the gallbladder was aspirated. Then, NOZ cells were injected with a 27-gauge syringe into the lumen of the gallbladder. Four weeks after orthotopic inoculation, all mice developed gallbladder tumors, lymph node metastasis, and distant metastasis in the liver mimicking human gallbladder cancer.

Conclusion

We established a novel cell line from human BTC and orthotopically engraftment model of BTC. These materials and methods are convenient and important tools for investigating the characteristics of BTCs and developing new therapeutic strategies.

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