MINI-REVIEW

Potential Therapeutic Targets for the Primary Gallbladder Carcinoma: Estrogen Receptors

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Abstract

Gallbladder carcinoma, the most frequent malignant neoplasm of the biliary tract system, has always been considered to feature late clinical presentation and diagnosis, limited treatment options and an extremely poor prognosis. In recent years, while the incidence of gallbladder cancer has appeared to be on the increase, the available treatment methods have not greatly improved survival of the affected patients. Thus, exploring new therapeutic targets for this devastating disease is an urgent matter at present. Epidemical studies have demonstrated that the incidence of gallbladder carcinoma exhibits a distinct gender bias, affecting females two to three times more than males, pointing to crucial roles of estrogen. It is well known that estrogen acts on target tissues by binding to estrogen receptors (ERs), which are mainly divided into three subtypes, ERα, ERβ and ERγ. ERα and ERβ appear to have overlapping but also unique even opposite biological effects. As important pathogenic mediators, ERs have been considered to relate to several kinds of tumors. In gallbladder carcinoma tissue, ERs have been shown to be positively expressed, and ERs expression levels are associated with differentiation and prognosis of this cancer. Nevertheless, the exact mechanisms of estrogen inducing growth of gallbladder carcinoma remain poorly understood. On the base of the current investigations, we deduce that estrogen participates in promotion of gallbladder carcinoma by influencing the formation of gallstones, stimulating angiogenesis, and promoting abnormal proliferation. Since ERs mediate the carcinogenic actions of estrogen in gallbladder, and therapy targeting ERs may provide new directions for gallbladder carcinoma. Therefore, it should be stressed that ERs are potential therapeutic targets for gallbladder carcinoma.

Keywords: Gallbladder carcinoma - estrogen - estrogen receptor - therapeutic target

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Introduction

Gallbladder carcinoma (GBC), firstly described in 1777 (Nevin et al., 1976), as a subtype of biliary tract cancer arises from gallbladder mucosa epithelia. It is a relatively rare malignancy but the most frequent malignant neoplasm of the biliary tract system and the fifth to six of digestive tract (Wistuba and Gazdar, 2004; Shaffer, 2008). More than 200 years later, when it comes to GBC, the impression to us still is late clinical diagnosis, lack of effective treatment methods and an extremely poor prognosis. Gallstones, inflammation, gender, aging, and obesity considered as high risk factors for GBC have been consistent in most of relevant reports (Hsing et al., 2007; Venniyoor, 2008; Boutros et al., 2012; Srivastava et al., 2012;Stinton and Shaffer, 2012). At present, complete surgical resection is the most effectively curative measure for GBC (Aretxabala et al., 2006; Furuse, 2008). Yet, majority of patients with GBC were in the advanced stage and missed the best treatment times of surgery, resulting in an overall dismal survival (Ito et al., 2004; Cho et al., 2010). Furthermore, incidence of GBC appears to ascent in recent years. Therefore, exploring new treatment targets for GBC is an urgent matter at present.

Epidemiological studies displayed that incidence of GBC exhibited distinct gender biasness, which affected females two to three times than males (Lazcano-Ponce et al., 2001; Gabbi et al., 2010). This gender predominance indicated that estrogen may be a possible promoter of carcinogenesis in gallbladder. Estrogen as the primary female sex hormone is the members of the family of steroid hormones, including estradiol (the most active one), estriol, and estrone. Most of estrogen is produced by the ovary, corpus luteum and placenta in female and the testis in male and small proportion of estrogen is synthesized by other tissues, such as the liver and breast,

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Table 1. The Comparison Between ERα and ERβ

Item	ERα	ERβ
The order of discovery	Firstly, the classic one, in 1980s	Secondly, in 1990s
The location in the chromatin	6q25.1	14q22–24
The location in the cell	Almost in the nuclear of the cell	Both in the nuclear and cytoplasm
The molecular structure	AF-1, weaker in activity	AF-1,the higher activity
The distribution of Tissue	uterine,breast, placenta,central nervous system,cardiovascular system,bone tissue	prostate, testis, ovarian, pineal thyroid pancreas, gallbladder skin lymphoid tissue of the urethra, erythrocyte
Affinity to the ligands	high	low
physiological functions	Fertility, mammary ,development, lactation	Efficiency of ovulation
Function in tumor	Promotion,	Inhibition, protection
Response to tamoxifen	Both agonists and antagonisms	Pure agonist effects

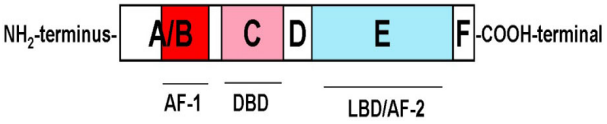

Figure 1. The Basic Structure of ER

where it may have localized effects (Wang et al., 2009). Estrogen plays critical roles in numerous physiological processes, including menstrual cycle, modulation of bone density, brain function, and cholesterol mobilization (Wang et al., 2009). In the pathological circumstances, estrogen can also influence a series of hormone-dependent diseases, such as breast, endometrial, and ovarian cancers, as well as osteoporosis. It is well known that all of the various biological effects of estrogen are mediated by binding to the specific estrogen receptors (ERs), which belong to the nuclear receptor superfamily, a family of ligand-regulated transcription factors (Evans,1988). In fact, ERs have been detectable in a range of human tumor tissues, such as breast (Yamaguchi and Hayashi, 2009; Welsh et al., 2012), the alimentary tract (Sica et al., 1984), melanoma (Fisher et al., 1976), endometrial (Wallace et al., 2010), ovarian (Spillman et al., 2010), pancreas (Greenway et al., 1981), biliary tract (Yamamoto et al., 1990; DeMorrow, 2009; Mancino et al., 2009; Park et al., 2009; Isse et al., 2010; Gupta et al., 2012; Hunsawong et al., 2012). Thereby, ERs may be the possible carcinogenic factors. In gallbladder cancer, ERs have been detected to be positive expression as well. Nevertheless, the specific details among estrogen, ERs and GBC remain to be determined. In present review, we analyzed the clinical implication of ERs expression in GBC, and deduced the possible mechanism of estrogen inducing GBC on the base of the present investigations, so as to explore new avenues for the therapy.

Estrogen Receptors

According to the subtypes, ERs were divided into ERα, ERβ, and ERγ (Hawkins et al., 2000). Most present studies about ERs mainly centered on ERα and ERβ. ERγ was only investigated in the teleost fish and vertebrates (Hawkins et al., 2000). ERα and ERβ are products of distinct genes and appear to have overlapping but unique even opposite biological effects. The comparison between ERα and ERβ is in the Table 1. Since their similar structure, the molecular mechanisms of the two receptors are similar. Both ERα and ERβ have five distinguishable domains, named the A/B, C, D, E and F domains, respectively (Figure 1). A/B domains, the most variable regains of ERs, contain an AF-1 (activation function regain-1), which mediated the ligand-independent tranactivation function and determined the promotor and cell specific activity. Due to the differences of the AF-1, the two receptors exhibited distinctive responses to the synthetic anti-estrogens tamoxifen, which is partial agonist for ERα but act as pure antagonists for ERβ. And also because of the differences in the AF-1, the transcription activity of the two receptors differs. DNA-binding domain (DBD) is located in the C-domain, which involved in specific DNA binding and receptor dimerization, where ERα and ERβ share a high degree of sequence identity. Thus, it is not surprising that both receptors bind estrogen responsive elements (EREs) with similar specificity and affinity. The E-domain contains a hormone-dependent activation function (AF-2) and the ligand-binding domain (LBD). The LBD are highly conserved and both receptors display similar affinities for the endogenous estrogen. There is a flexible hinge regain in D-domain between the DBD and LBD, and appears to be important for nuclear translocation. The functions of F-domain remain undefined. These several domains interact synergistically to ensure ERs biological effects.

ERs binding with the ligands (17β-estradiol,E2), are induced moleculor conformation changes, which lead to dimerization, protein–DNA interaction and other transcription factors, and then the formation of the preinitiation complex, and ultimately regulates the transcription of the target genes. Nevertheless, ERβ exhibits an antagonistic effect on ERα.When co-expressed with ERα, ERβ can inhibit ERα-mediated transcriptional activity (Lindberg et al., 2003). Additionally, the opposing actions between ERα and ERβ showed in the regulation of the cyclin D1 expression (Liu et al., 2002). Generally speaking, ERα shows a promotion in tumor development, but ERβ as a possible tumor suppressor.

Expression Status of ERs in GBC

In the past decades, most of the investigations about the associations between estrogen and GBC have focused on the ER expression in resected or biopsied specimens of GBC (Ohnami et al., 1988; Nakamura et al., 1989; Yamamoto et al., 1990; Ko et al., 1995; Roa et al., 1995; Sumi et al., 2004; Park et al., 2009). In the 80's and early 90's, several researchers have identified that ERs expression was positive in GBC (Stedman et al., 1980; Nakamura et al., 1989; Yamamoto et al., 1990). Recently,

a study, applied in immunohistochemistry, found the expression levels of ERs were significantly higher in GBC than chronic cholecystitis (Gupta et al., 2012). Yamamoto et al. (1990) have evaluated ERs expression both in benign and malignant tissues of gallbladder, including cholelithiasis, epithelia polyp, adenoma, and adenocarcinoma by immunohistochemistry, and the results showed that ERs was positive-expressed in each stage of the gallbladder disease, and presence of ERs was correlated to metaplasia of the gallbladder mucosa. Sumi et al. (2004) analyzed 26 GBC samples, and reported that ERβ expression between non-cancerous and cancerous regions was obviously different. Park et al. (2009) investigated the expression of ERα, ERβ and progesterone receptor in 30 specimens of gallbladder adenocarcinoma tissues after radical resection. The results were that ERα and progesterone receptor were negative, but 73.3% of the specimens (22 of 30 cases) were positive for ERβ. They also found that ERβ expression was correlated with tumor differentiation and prognosis. Nakamura et al. (1989), in patients with GBC, found ERs expression more in moderately (50%) to poorly (100%) differentiated tumors than in well-differentiated tumors (44%). However, there were some reports which failed to detect the expression of ERs in the GBC (Shukla et al., 2007; Albores-Saavedra et al., 2008).

Possible Mechanisms of Estrogen Promotion of GBC

A report (Cirillo et al., 2005) from American has assessed the effects of estrogen therapy on the healthy postmenopausal women by clinical randomized control trials. The sample size involved in 22579 participants, aged 50 to 79 years. They found that incidence of gallbladder disease was evidently increased compared with the control groups. Thus, a conclusion that estrogen participate in the occurrence of gallbladder diseases can be easily obtained.

Estrogen is capable of inducing cell proliferation and anti-apoptosis (Isse et al., 2010). Furthermore, it also can promote the metastasis of the tumor by inducing expression of a set of metastasis genes (Stossi et al., 2004). Actually, the critical roles of estrogen in the breast cancer have been well established, and produced heartening fruits, such as the widespread application of anti-estrogen tamoxifen (Mann et al., 2001; Honma et al., 2008). Therefore, estrogen was known as the promoting factor in the initiation and progression of tumor, including GBC. Although estrogen is closely related to GBC, the precise mechanism remains obscure. According to limited present investigations, we deduce the possible mechanism by which estrogen induce the occurrence and development of GBC.

Estrogen and Formation of Gallstones

Epidemical and correlative investigations considered gallstones as the high risk factors for GBC (Venniyoor, 2008; Stinton and Shaffer, 2012). Gallstones are present in approximately 70% to 90% of patients with GBC (Ahrens et al., 2007; Hsing et al., 2007; Shaffer, 2008; Boutros et al., 2012). Clinical observation found that patients with a long history of gallstones more likely developed into gallbladder cancer. This may be caused by the long-term mechanical irritation of gallstones to the gallbladder mucosa, leading to dysplasia, atypical hyperplasia and finally advancing to carcinoma.

Several studies demonstrated that estrogen can promote the formation of cholesterol gallstones (Petitti, 1988; Everson et al., 1991; Wang et al., 2004). The gonadectomized mice with subcutaneously implanted with pellets releasing E2 and fed a lithogenic diet for 12 weeks, had exhibited a E2 dose-dependent increase of gallstones, and ICI 182, 780 (estrogen receptor inhibitor) can blocked this effects (Wang et al., 2004). At molecular levels, the "estrogen- ERα-SREBP-2" pathway can cause biliary cholesterol hypersecretion (Wang et al., 2006). Also, estrogen can stimulate the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in hepatic cholesterol biosynthesis (Reichen et al., 1987; Wang et al., 2006). Additionally, estrogen can enhance intestinal cholesterol absorption (Duan et al., 2006). All of these can contribute to biliary cholesterol hypersecretion and cholesterol supersaturation of bile, which significantly enhance the formation of cholesterol gallstones. Of note is that the effects of E2 were receptor- and dose- dependent. Furthermore, the effects of estrogen on promoting the formation of gallstone were mediated by the ERα, not ERβ (Wang et al., 2004; Wang et al., 2006). Taken together, it is naturally hypothesized that estrogen participated in gallbladder cancer pathogenesis partly by "estrogen- ERα-gallstone-gallbladder cancer" pathway.

Estrogen and Angiogenesis

Inducing angiogenesis is one of the ten hallmarks of cancer (Hanahan and Weinberg, 2011). Several studies have demonstrated that estrogen has profound effects on angiogenesis. In gene levels, estrogen can regulate the gene transcription of vascular endothelial growth factors (VEGF) which are essential to neovascularization (Mueller et al., 2000). In vitro and mice model, estrogen can stimulate angiogenic activity in human umbilical vein endothelial-cells. In thyroid tumor microenvironment, estrogen via ER and VEGF can induce a proangiogenic endothelial cell phenotype (Kamat et al., 2011). In order to investigate how estrogen affects the growth and development of breast cancer, a significant experiment has been done. Using different mouse models, in which ER-negative cancer cells were grafted subcutaneously, it was observed that estrogen increased intratumoral vessel density and promoted tumor growth. These estrogen-induced effects were completely blocked in ERα-deficient mice, suggesting a critical role of ERα in the process (Pequeux et al., 2012). In the biopsy samples and cell lines of cholangiocarcinoma, estrogen can enhance VEGF and their specific receptors expression and the proliferation mediated by estrogen was thought to be associated with VEGF (Mancino et al., 2009). Thus, estrogen may promote the tumor development by inducing the formation of newborn vessels. Nevertheless, this works have not been done in the GBC. Similar to other cancers, GBC also

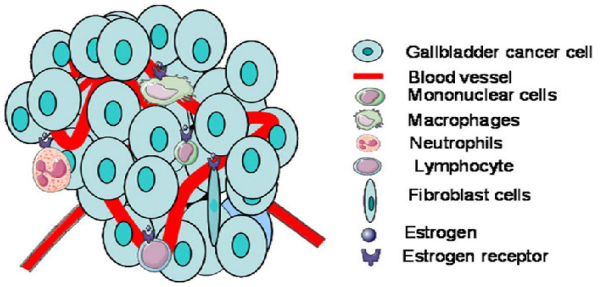

Figure 2. Estrogen Acts on GBC Tissue by Binding with ER in the Microenvironment Cell, Such as Macrophages, Lymphocyte, Fibroblast Cell, and So on

needs abundant oxygen and nutrient, and angiogenesis is critical to GBC development. Most importantly, VEGF is high expression in GBC tissue (Quan et al., 2001; Giatromanolaki et al., 2003; Sun et al., 2011). Therefore, we can speculate that estrogen-ER-VEGF-angiogenesis pathway may be the partial mechanism of estrogen involved in the progression of GBC.

However, how did estrogen work in the GBC tissue where ERs failed to be detected? A study (Iyer et al., 2012), which investigated the role of estrogen in the ER-negative breast cancer tissue, found ER existed in the cells of tumor microenvironment (such as endothelial cell, neurons, immune cells (macrophage, lymphocyte), fibroblasts, and so on), not the parenchymal tumor cells. Therefore, a possibility is that ER is positive in the microenvironment cells of GBC. The biological effects of estrogen to the GBC tissue can be mediated by these ERs (Figure 2). When estrogen binding with ERs, microenvironment cell in GBC tissue can secrete some cytokines, such as VEGF, and then regulate the growth of GBC.

Estrogen and abnormal proliferation

Abnormal proliferation is another important characteristic of tumor (Hanahan and Weinberg, 2011) .The effects of promoting proliferation and ant-apoptosis of estrogen have been investigated (Alvaro et al., 2003; Mancino et al., 2009; Isse et al., 2010). In mammary gland tissue, the promoting proliferation of estrogen has well been established (Lydon, 2010). The female mammary gland has undergone tightly choreographed process of cell proliferation from puberty to menopause, in which, a miscue can cause the mammary tissue to develop into cancer (Lydon, 2010). This indicated that tumor may come from the abnormal proliferation. In the biliary tract system, estrogen and their receptors can modulate the proliferation of cholangiocyte (Alvaro et al., 2000). In vivo and vitro experiments, estrogen induced cholangiocyte proliferation by activating the Src/Shc/ERK pathway, and the effects can be inhibited by antiestrogen, tamoxifen or ICI 182,780 (Alvaro et al., 2003). In addition, a synergistic effect of nerve growth factor, insulin-like growth factor, and EBP50 (inradixin moesin (ERM) binding phosphoprotein50) with estrogen participated the proliferation of cholangiocarcinoma cell (Alvaro et al., 2006; Fouassier et al., 2009). However, relevant investigations have not been done in GBC. Since the similarity in tissue origination between GBC and cholangiocarcinoma, we can speculate that inducing the anomalous proliferation by estrogen may be another possible mechanism of GBC in the onset and progression.

Discussion Points

To date, the knowledge on estrogen, ER with GBC is limited. Several studies suggested estrogen participated in the development of GBC, despite the precise details remained unclear (Chen and Huminer, 1991; Fernandez et al., 2003; Cirillo et al., 2005). According to present relevant studies, we deduce that estrogen promote GBC by influencing the formation of gallstones, inducing the angiogenesis, and promoting the proliferation of cell. Of course, all of these hypothesizes need to be confirmed in future studies. ERs as profound mediators of biological effects of estrogen, expressed positively in GBC tissue, have important clinical and pathological implications. In the gene level, the single nucleotide polymorphism (SNP) of ER has been identified to be correlated to the risk of GBC (Park et al., 2010; Gupta et al., 2012).In the biopsy specimens and cell lines, some reports have revealed that ERs expression was correlated to the differentiation and prognosis of patients with GBC. A study showed that more ERs expression in moderately and poorly differentiated GBC tissue than well differentiated lesions (Nakamura et al., 1989). In addition, ERβ, not ERα, may be an essential prognostic factor for GBC. In fact, expression of ERβ has been considered as an independent factor of prognosis in pleural mesothelioma (Shukla et al., 2007). In the prostate cancer, ERβ was as the prognostic factor as well (Albores-Saavedra et al., 2008). A follow-up study (Park et al., 2009) to patients with GBC has been done and the results demonstrated that the five year survival of ERβ-positive and ERβ-negative patients with GBC was 53.3% and 31.1% respectively, and the difference was statistically significant. Furthermore, the loss expression of ERβ at the invasive front was associated with malignant properties of GBC such as lymph node metastasis, advanced stage, lower histological differentiation and an extremely poor prognosis (Sumi et al., 2004). Besides, the detection of ERs expression can guide the endocrine therapy of GBC. Actually, endocrine therapy may be to disrupt estrogen receptor activity, which is suitable for patients with strong ER-expression, rather than weak, especially even absent ER-expression. It is well known that ER in mammary carcinoma has been investigated very deeply, and anti-estrogen therapy has been used as a clinical routine method. Due to the widespread application of anti-estrogen tamoxifen, the survival rate of breast cancer has increased drastically (Chia et al., 2005; Dunnwald et al., 2007). Similarly, ER as a potential therapeutic target for GBC may provide a novel direction for treatment.

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