INTRODUCTION

Accumulating data demonstrate that the incidence and prognosis of many types of diseases differ depending on patients’ sex/gender. The sex/gender disparity may be due to genetic differences on the X chromosome, differences in physiological conditions such as hormone levels, and other factors (Spatz et al., 2004; Bottarelli et al., 2007; Scosyrev et al., 2009; Klinge, 2012; Gabriele et al., 2016). It should be noted that ‘sex’ and ‘gender’ are not mutually exclusive terms (Clayton and Tannenbaum, 2016; Heidari et al., 2016). In general, ‘sex’ is used for biological expression, such as gene expression or hormone-related symptoms, while ‘gender’ is used when lifestyle, behavior, and environment are reflected (Clayton and Tannenbaum, 2016; Heidari et al., 2016; Pelletier et al., 2016). Sex/gender-biased diseases include autoimmune diseases, neurodegenerative diseases, and cardiovascular diseases (Skavdahl et al., 2005; Cantuti-Castelvetri et al., 2007; Gleicher and Barad, 2007; Haley et al., 2010). Diseases showing sex/gender disparities also include several types of cancers, including liver cancer, melanoma, and thyroid cancer (Bray et al., 2018). Liver cancer and melanoma have lower incidence rates and better prognosis in females than in males (Bray et al., 2018; Smalley, 2018). In contrast, the incidence rate of thyroid cancer is three times higher in females than in males, even though there is not much difference in mortality.

Sex/gender disparity has been shown in the incidence and prognosis of many types of diseases, probably due to differences in genes, physiological conditions such as hormones, and lifestyle between the sexes. The mortality and survival rates of many cancers, especially liver cancer, differ between men and women. Due to the pronounced sex/gender disparity, considering sex/gender may be necessary for the diagnosis and treatment of liver cancer. By analyzing research articles through a PubMed literature search, the present review identified 12 genes which showed practical relevance to cancer and sex disparities. Among the 12 sex-specific genes, 7 genes (BAP1, CTNNB1, FOXA1, GSTO1, GSTP1, IL6, and SRPK1) showed sex-biased function in liver cancer. Here we summarized previous findings of cancer molecular signature including our own analysis, and showed that sex-biased molecular signature CTNNB1High, IL6High, RHOAHigh and GLIPR1Low may serve as a female-specific index for prediction and evaluation of OS in liver cancer patients. This review suggests a potential implication of sex-biased molecular signature in liver cancer, providing a useful information on diagnosis and prediction of disease progression based on gender.

Key Words: Liver cancer, Sex/gender, Overall survival, Gene expression, Molecular signature

Sex-Biased Molecular Signature for Overall Survival of Liver Cancer Patients

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Abstract

Sex/gender disparity has been shown in the incidence and prognosis of many types of diseases, probably due to differences in genes, physiological conditions such as hormones, and lifestyle between the sexes. The mortality and survival rates of many cancers, especially liver cancer, differ between men and women. Due to the pronounced sex/gender disparity, considering sex/gender may be necessary for the diagnosis and treatment of liver cancer. By analyzing research articles through a PubMed literature search, the present review identified 12 genes which showed practical relevance to cancer and sex disparities. Among the 12 sex-specific genes, 7 genes (BAP1, CTNNB1, FOXA1, GSTO1, GSTP1, IL6, and SRPK1) showed sex-biased function in liver cancer. Here we summarized previous findings of cancer molecular signature including our own analysis, and showed that sex-biased molecular signature CTNNB1High, IL6High, RHOAHigh and GLIPR1Low may serve as a female-specific index for prediction and evaluation of OS in liver cancer patients. This review suggests a potential implication of sex-biased molecular signature in liver cancer, providing a useful information on diagnosis and prediction of disease progression based on gender.

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rate (Bray et al., 2018).

Liver cancer, the fourth leading cause of cancer death worldwide (Bray et al., 2018), shows a prominent sex/gender disparity. For liver cancer, the incidence is 3-5 times higher in males, while the mortality rates is twice as high in males than in females (Bray et al., 2018; Jung et al., 2019). The reasons for this sex/gender disparity are complex. Sex hormones, metabolic factors, and behavioral factors have been suggested as risk factors for liver cancer (Lin et al., 2013; Welzel et al., 2013; Kohi, 2016). Due to the pronounced sex/gender disparity, considering sex/gender may be necessary for the diagnosis and treatment of liver cancer (Guy and Peters, 2013).

Cancer is a complex disease and cancer genes do not act separately and deregulation of various genes from different pathways can lead to cancer initiation or progression (Hanahan and Weinberg, 2011). Gene signature or gene expression signature is a single or combined group of genes in a cell with different pattern of gene expression that occurs as a result of pathogenic condition. Many studies have been made to predict gene signatures related to cancer (van de Vijver et al., 2002; Allahyar and de Ridder, 2015) and produce informative genes or sub-networks by considering a predefined biological network (Babaei et al., 2013). Gene signature that can be applied for a broad range of cancers could be highly useful in research and clinical settings as a biomarker. The discovery of molecular signatures is proving to be a powerful tool for disease diagnosis and drug discovery.

The predictive effect of single gene biomarkers is not sufficiently specific (Zhao et al., 2019). When a cell becomes cancerous, the changes in the gene or protein expression patterns that causes the biological characteristics as a cancer cell occur. The cluster of genes or proteins representing these changes is called a cancer molecular signature (Nilsson et al., 2009; Sung et al., 2012). Significant clinical phenotypes resulting from these changes can be predicted by cancer molecular signature. They include the progression of diseases and the consequent increase in risk rates (Mehrabian et al., 2007; Hur et al., 2011), the response to drugs used to treat diseases and the resulting toxicity evaluation (Hines et al., 2010; Cohen et al., 2011; Xie et al., 2012), and the prediction of recurrence or death of diseases (Pittman et al., 2004; Bevelstad et al., 2007).

In this review, we summarized cancer molecular signature which reported previously and cancer biomarker candidates reflecting sex disparity. In addition, we described how we identified 12 sex-biased genes and their expression patterns in liver cancer patient. More importantly, we discussed that sex-biased molecular signature CTNNB1High and RHOAHigh and GLIPR1Low correlated with overall survival (OS) in liver cancer patients with sex-dependency.

**CANCER MOLECULAR SIGNATURE**

Recently, many significant cancer molecular signatures have been published (Tang et al., 2017; Erstad et al., 2018; So et al., 2018). This is due to the development of various omics capable of analyzing a large number of samples at the same time and bioinformatics capable of analyzing a database reflecting the research results and clinical information accumulated over a long period of time.

All 1207 differentially expressed genes (DEGs) between recurrent and non-recurring samples in colon cancer patients were analyzed (Xu et al., 2017). Through support vector machine (SVM) analysis and verification of gene expression profiling, molecular signatures including 15 genes (HES5, ZNF417, GLRA2, OR8D2, HOXA7, FABP6, MUSK, HTR6, GRIP2, KLRK1, VEGFA, AKAP12, RHEB, NRCPA00152, PMEPA1) were identified as indicators that inform the prognosis and recurrence risk in colon cancer patients (Xu et al., 2017).

Wang et al. (2019) analyzed 332 DEGs between normal ovarian tissue and ovarian cancer tissue and observed the associated prognosis. Sixty-four of them were significantly correlated with the OS of ovarian cancer patients, and five genes, IGF2, PEG3, DCN, LYPD1, and RARE1 were selected and screened to construct a 5-gene signature (Wang et al., 2019). As a result of clinical analysis, patients with low expressed 5-gene signature had significantly better OS compared to patients with the high expression (p=0.0004).

CD44-high and CD24-low cells not only express cancer stem cell-related genes, but also represent epithelial-mesenchymal transition (EMT) properties. When the relative expression of CD44 and CD24 was observed in the clinical samples of oral squamous cell carcinoma patients, CD44 expression was high in tumor tissues, but CD24 was significantly low. Therefore, CD44-high and CD24-low have high potential to be used as a molecular signature of cancer stem-like cells in oral squamous cell carcinoma (Ghuwalewala et al., 2016).

After neoadjuvant chemotherapy for operable gastroesophageal cancer, lymph node metastasis is known as the only proven variable that can predict the prognosis (Smyth et al., 2016). In the high- and low-risk groups of OS in The Medical Research Council Adjuvant Gastric Infusional Chemotherapy trial samples, 7-gene signature (CDH1, ELOVL5, EGFR, PIP5K1B, FGF1, CD44v8.10, TBCE) could independently predict the patient’s prognosis (Smyth et al., 2018). These results suggest that stratification of patients using this 7-gene signature may help in postoperative chemotherapy selection.

In hepatocellular carcinoma (HCC), there have been reported on several molecular signatures related to cancer growth and malignancy. Molecular signature including five genes, ANGPT2, NETO2, ESM1, N4R4A1 and DLU4, can be showed HCC growth, invasion into blood vessels, recurrence of cancer, and degree of intrahepatic metastasis (Villa et al., 2016). MicroRNA signature related to intravenous invasion and metastasis can be used to predict disease free survival and OS: highly upregulation of miR-219, miR-207 and miR-338, and extremely downregulation of miR-34, miR-30, and miR-148 (Budhu et al., 2008). The gene signature reflecting abnormal DNA methylation in HCC, SCAND3, SGIIP1 and PI3, can be used to determine the risk of recurrence in patients with resected early-stage HCC (Qiu et al., 2017). Molecular signatures in HCC can contribute to the development of targeted treatment regimens. In addition, more accurate prognosis can be predicted after treatment, selective and intensive monitoring of patients with poor prognosis can be performed, and clinical trial design can be improved, such as subdividing diseases with similar advanced stages (Erstad et al., 2018).

**SEX-BIASED CANCER BIOMARKER CANDIDATES**

Men and women show distinct sex/gender-biased differ-
ences in various areas, including growth rate and lifespan, metabolism and immune mechanisms, which are affected by carcinogenesis and cancer progression, treatment mechanisms, and survival. This is caused by sexual dimorphism that occurs due to sex-biased differences including genetic and epigenetic mechanisms as well as sex hormones that circulate in the body and induce sex disparities. Sex disparity have also been demonstrated in the expression of biomarkers that predict prognosis and diagnosis of cancer and the rate and pattern of cancer metastasis, and the response to various trials of treatment in different cancer types (Pal and Hurria, 2010; Mervic, 2012).

There are sex disparities in the epigenetic mechanisms of autosomal and sex chromosome genes (El-Maarri et al., 2007; Tobi et al., 2009; Reviewed in Yuasa, 2010). And impairment of epigenetic regulation is known as an important mechanism for the incidence and progression of cancer (Tobi et al., 2009). DNA methylation is the most extensively applied epigenetic marker that represented by sex-biased expression of gene such as DNA methyltransferases (DNMTs) (Njugent et al., 2015; Mosley et al., 2017). Reizel’s group reported for DNA methylation in the liver, where males are hypomethylated compared to females due to testosterone exposure, and demonstrated that it was regulated by DNA methylation with sex disparity (Reizel et al., 2015).

One of the most striking differences of epigenetics in male and female is the inactivation of additional X-chromosomes in female cells (Rubin et al., 2020). Some long non-coding RNA (IncRNA), such as five prime to Xist (FTX), and a lot of epigenetic modifiers located on X chromosome, such as lysine demethylases KDM6A and KDM5C, are concerned with inactivation of X chromosome and has been known as presumed tumor suppressors in HCC (Wijchers and Festenstein, 2011; Liu et al., 2016). They are highly expressed in female HCC patients and suppress the proliferation and invasion of HCC cells (Xu et al., 2008; Liu et al., 2016; Snell and Turner, 2018). Their expressions correlate positively with cancer survival and decrease the risk of liver cancer in females.

Sex-biased metabolic pathways also influence cancer progression and treatment mechanisms. In the overall metabolic mechanisms, the expression of molecules involved in carbohydrate and amino acid metabolism is increased in men, and the expression of molecules involved in fatty acid metabolism is increased in women (Mittelstrass et al., 2011; Garcia-Herrejós et al., 2011; Harish et al., 2011; Ippolito et al., 2017). In spite of the need for higher mitochondrial activity, female mitochondria produce less reactive oxygen species (ROS), one of the causes of cell damage (Borras et al., 2003; Harish et al., 2013). Fundamental sex disparities in metabolic pathways, such as the use of nutrients in the body and mitochondrial function, can lead to sex-biased differences in the incidence and progression of cancer, and further, the mechanism of application of chemotherapy.

In general, females have a stronger and more adaptable immune response system than males (Cook et al., 2009; Klein and Flanagan, 2016). These immune responses with sex disparity may contribute to differences in cancer progression and mortality according to sex. Females have a greater number of neutrophils and macrophages and more active phagocytosis than males due to the inhibited secretion of inflammatory cytokines by estrogen (Scotland et al., 2011; Laffont et al., 2017). These researches have suggested that estrogen decrease the risk of cancer in females. The X chromosome contains the largest number of immune-related genes in the entire human genome such as FOXP3 and CD40L (Fish, 2008; Libert et al., 2010; Pinheiro et al., 2011; Bianchi et al., 2012). Given the differences of expression of sex chromosome genes in male and female, X-linked immunoregulatory genes and sex hormones are expected to play an important role in mediating sex-biased immune response.

Sex hormones additionally affect sex disparity in the angiogenesis process in cancer via different expression of circulating angiogenic factors by intrinsically different endothelial cells (ECs) (Addis. et al., 2014). As shown in Evanson’s study, platelet-rich plasma of adult females has more pro-angiogenic factors, including vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived growth factors (PDGFs), and so on (Evanson et al., 2014). On the other hand, several angiogenic growth factors, such as basic fibroblast growth factor (bFGF), transforming growth factor-beta 1 (TGF-β1) and tumor necrosis factor-alpha (TNF-α), were higher expressed in male plasma (Xiong et al., 2018). However, there is no clear elucidation of how certain growth factors and cytokines have sex-biased expression, and how this sex disparity translates into differential angiogenesis signals and functions (Rubin et al., 2020). These findings suggest the need for analysis of correlation between pro- or anti-angiogenic factors and sex.

The sex-biased molecular differences induced by the various causes of sex disparity mentioned earlier were identified via systematic evaluation of omics and big data analysis. These identified molecules applied as sex-biased potential biomarker candidates (Li et al., 2018; Shin et al., 2019).

Efforts have been made to elucidate sex-biased gene expression and functions in liver cancer. Wu et al. (2019) suggested CDK1, CCNB1, CYP3A4 and SERPIN4A as sex-biased cancer molecular signature which have been identified as DEGs with sex dimorphism in HCC via gene expression profiling, the most frequent type of liver cancer (Wu et al., 2019). Phosphoglucomutase-like protein 5, encoded by the PGM5 gene, was shown to have potential as a male-specific prognostic biomarker reflecting overall survival (OS) probability in liver cancer (Jiao et al., 2019). However, there is no known marker indicating the risk of liver cancer in female patients.

Although sex-biased differences in HCC development risk are well recognized (Setawain et al., 2016; Wu et al., 2018; Yu et al., 2019; Rich et al., 2020), the prognosis between sexes remains unclear. Sex hormones are expected to play an important role in the sex disparity of malignancy. Androgen/androgen receptor signaling is known to be involved in tumor promotion as well as estrogen/estrogen receptor signaling is involved in tumor protection in mouse models (Li et al., 2012). Function of sex hormones in HCC has been suggested as repressor of interleukin-6 and STAT3 inactivation (Naugler et al., 2007; Hou et al., 2013). Therefore, in order to understand why male and female patients show difference in HCC development and prognosis, sex-biased molecular signature would be required to predict and evaluate the prognosis of liver cancer.

We conducted a PubMed search (https://www.ncbi.nlm.nih.gov/pubmed/) for papers describing sex-biased genes known to be related to cancer. Using five keywords, ‘cancer,’ ‘malignancy,’ ‘sex,’ ‘gender,’ and ‘gene,’ the PubMed search resulted in 598 related papers. After carefully checking these papers,
12 cancer-related genes that showed sex-biased differences were selected for further analysis (Table 1). These genes code for proteins with critical roles in the cell cycle, cellular differentiation, regulation of cell death and growth, or cancer development processes such as angiogenesis and metastasis.

**SEX/GENDER-BIASED OVERALL SURVIVAL PROBABILITY OF LIVER CANCER PATIENTS WITH SELECTED CANCER-RELATED GENES**

To alleviate the aggressive progression of liver cancer in women, timely and appropriate diagnosis that reflects individual differences, such as sex/gender disparities, is essential. Identified 12 genes which showed practical relevance to cancer and sex disparities via a PubMed literature search were investigated the correlation between expression of these genes and survival.

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**Table 1. List of previously reported cancer biomarker candidates with sex disparity identified by a PubMed search**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Gene name</th>
<th>Sex-biased function in cancer</th>
<th>References</th>
</tr>
</thead>
</table>
| BAP1   | BRCA1 Associated Protein 1 | - Regulation of cell cycle, cellular differentiation, and DNA damage 
- More frequent mutation in female-derived HCC | Li et al., 2018; Masoomian et al., 2018 |
| BRUCE (BIRC6) | BIR Repeat-Containing Ubiquitin-Conjugating Enzyme | - Regulation of tumor cell death 
- Higher levels of expression specific in female | Salehi et al., 2017 |
| CTNNB1 | Catenin Beta 1 | - Regulation of cell growth and adhesion between cells 
- More frequent mutation in male liver cancer patients | Xia et al., 2006; Li et al., 2018 |
| FOXA1 | Forkhead Box Protein A1 | - Regulation of apoptosis and cell cycle 
- Significantly expressed higher in female HCC | Li et al., 2012, 2017 |
| GLIPR1 | Glioma pathogenesis-related protein 1 | - Regulation of cell growth and chemokine secretion 
- Lower expressed specific in male thyroid cancer patients | Li et al., 2011; Zhang et al., 2015 |
| GSTO1 | Glutathione S-transferase omega-1 | - Protection of normal cells against damage induced by carcinogens in HCC 
- Significantly associated with overall survival in HCC patients | Niu et al., 2009; Qu et al., 2015 |
| GSTP1 | Glutathione S-transferase pi-1 | - Strong correlation between inflammation and cancer 
- Low expression reduced risk of cancer in female liver cancer patients | Naugler et al., 2007; Liu and Liu, 2014; Kumari, et al., 2016 |
| IL6 | Interleukin 6 | - Suppression of cancer metastasis 
- Significantly expressed highly in female pituitary tumors patients | Shirasaki et al., 2001; Yaron et al., 2015 |
| KISS1R | Kisspeptin receptor 1 | - Promotion of tumor cell proliferation and metastasis 
- Significant reduction in survival of the entire cohort and across gender subgroups | De Rienzo et al., 2016 |
| PER1 | Period 1 | - Regulation of cell cycle and promotion of DNA repair 
- Higher expression in female colon cancer patients | Wang et al., 2015 |
| RHOA | Ras Homolog Family Member A | - Regulation of mRNA splicing 
- Higher expression significantly correlated with sex specific to male HCC patients | Graveley, 2000; Zhang et al., 2016 |
and cancer malignancy by Kaplan-Meier (KM) plot analysis using clinical data from liver cancer patients in The Cancer Genome Atlas (TCGA) database. Among the 12 sex-biased genes, 7 genes (BAP1, CTNNB1, FOXA1, GSTO1, GSTP1, IL6, and SRPK1) showed sex-biased function in liver cancer (Table 1), suggesting that cancer biomarker candidates with sex disparity may be reliable in liver cancer.

The repositories in TCGA datasets of male and female liver cancer patients with available survival data were analyzed for the 12 genes. In order to obtain survival data for male and female liver cancer patients, the transcriptomic dataset in TCGA (version 2016_01_28; https://cancergenome.nih.gov/) was analyzed and plotted to KM plots using The Kaplan Meier Plotter (http://kmplot.com). Male (N=246) and female (N=118) liver cancer patients were divided using the auto-select best cutoff criteria. The cutoff values for high and low expression for each gene were as follows: catenin beta 1 (CTNNB1, female, 7932; male, 10396), IL6 (female, 3; male, 2), glioma pathogenesis related 1 (GLIPR1, female, 153; male, 399), and Ras Homolog Family Member A (RHOA, female, 13302; male, 13631). A detailed description of the patient populations is given in Table 2.

Three out of the 12 genes, CTNNB1, IL6 and GLIPR1, showed sex disparity in OS probability in liver cancer patients (Fig. 1A-1C). Male liver cancer patients with relatively high expression of CTNNB1 showed a higher OS probability than did male liver cancer patients with low expression of CTNNB1 (HR=0.67). In contrast, female liver cancer patients with relatively high expression of CTNNB1 showed a lower OS probability and higher risk than did female liver cancer patients with low expression of CTNNB1 (HR=1.91, logrank p=0.026). These data indicate that there is a positive correlation between CTNNB1 expression and OS probability in male liver cancer patients, but there is a negative correlation in female liver cancer patients.

As shown in Fig. 1B, male liver cancer patients with relatively high expression of IL6 showed a significantly higher OS probability than did male liver cancer patients with low expression of IL6 (HR=0.61, logrank p=0.029). In females, liver cancer patients with relatively high expression of IL6 showed a significantly lower OS probability than did patients with low expression of IL6 (HR=2.34, logrank p=0.018). These results suggest a positive correlation between IL6 expression and OS probability in male liver cancer patients, but a negative correlation in female liver cancer patients, as in the case for CTNNB1. These data show that female liver cancer patients with higher expression of IL6 had poorer prognoses than those with lower levels of IL6.

In the analysis of GLIPR1 (Fig. 1C), male liver cancer patients with relatively high expression of GLIPR1 showed a

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Gene name</th>
<th>Male HR (95% CI)</th>
<th>Male Logrank p</th>
<th>Female HR (95% CI)</th>
<th>Female Logrank p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP1</td>
<td>BRCA1 Associated Protein 1</td>
<td>0.77 (0.49-1.2)</td>
<td>0.25</td>
<td>1.47 (0.85-2.54)</td>
<td>0.17</td>
</tr>
<tr>
<td>BRUCE (BIRC6)</td>
<td>BIR Repeat-Containing Ubiquitin-Conjugating Enzyme</td>
<td>0.75 (0.47-1.19)</td>
<td>0.22</td>
<td>0.83 (0.48-1.45)</td>
<td>0.52</td>
</tr>
<tr>
<td>CTNNB1*</td>
<td>Catenin Beta 1</td>
<td>0.67 (0.39-1.14)</td>
<td>0.14</td>
<td>1.91 (1.07-3.4)</td>
<td>0.026</td>
</tr>
<tr>
<td>FOXA1</td>
<td>Forkhead Box Protein A1</td>
<td>0.71 (0.44-1.15)</td>
<td>0.16</td>
<td>0.68 (0.35-1.34)</td>
<td>0.26</td>
</tr>
<tr>
<td>GLIPR1*</td>
<td>Glioma pathogenesis-related protein 1</td>
<td>1.51 (0.97-2.36)</td>
<td>0.069</td>
<td>0.54 (0.30-0.96)</td>
<td>0.034</td>
</tr>
<tr>
<td>GSTO1</td>
<td>Glutathione S-transferase omega-1</td>
<td>0.67 (0.38-1.16)</td>
<td>0.15</td>
<td>0.76 (0.41-1.45)</td>
<td>0.41</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Glutathione S-transferase pi-1</td>
<td>0.83 (0.53-1.29)</td>
<td>0.41</td>
<td>0.59 (0.32-1.1)</td>
<td>0.091</td>
</tr>
<tr>
<td>IL6*</td>
<td>Interleukin 6</td>
<td>0.61 (0.38-0.96)</td>
<td>0.029</td>
<td>2.34 (1.13-4.83)</td>
<td>0.018</td>
</tr>
<tr>
<td>KISS1R</td>
<td>Kisspeptin receptor 1</td>
<td>1.38 (0.86-2.21)</td>
<td>0.17</td>
<td>1.68 (0.96-2.93)</td>
<td>0.066</td>
</tr>
<tr>
<td>PER1</td>
<td>Period 1</td>
<td>0.39 (0.25-0.62)</td>
<td>3.6e-05</td>
<td>0.51 (0.28-0.93)</td>
<td>0.025</td>
</tr>
<tr>
<td>RHOA*</td>
<td>Ras Homolog Family Member A</td>
<td>1.65 (1.06-2.56)</td>
<td>0.026</td>
<td>1.86 (1.06-3.27)</td>
<td>0.029</td>
</tr>
<tr>
<td>SRPK1</td>
<td>Serine-arginine protein kinase 1</td>
<td>1.74 (1.1-2.75)</td>
<td>0.017</td>
<td>2.15 (1.22-3.8)</td>
<td>0.0066</td>
</tr>
</tbody>
</table>

*Gene with a sex disparity in the correlation between expression and overall survival in liver cancer patients.
*Gene with a high HR value in both male and female liver cancer patients, but prominent sex differences with longer survival periods.
lower OS probability than did male liver cancer patients with low expression of GLIPR1 (HR=1.51). Female liver cancer patients with relatively high expression of GLIPR1 showed a significantly higher OS probability than did female liver cancer patients with low expression of GLIPR1 (HR=0.54, logrank P=0.034). Unlike CTNNB1 and IL6, there is a negative correlation between GLIPR1 expression and OS probability in male liver cancer patients, whereas a positive correlation exists in female liver cancer patients.

There was no apparent sex difference between males and females in HR for RHOA (HR=1.65 in males and HR=1.86 in females). Of note, the difference in OS probability between high RHOA expression and low RHOA expression was more drastic in male liver cancer patients than in female liver cancer patients (Fig. 1D). Taken together, our analysis demonstrates a sex-dependent correlation between OS probability in liver cancer patients and expression levels of CTNNB1, IL6, GLIPR1, and RHOA.

CTNNB1 codes for β-catenin, a known key molecule in canonical WNT signaling (Behari, 2010).
pathway is a critical regulator in cancers including liver cancer, and β-catenin plays an important role in liver regeneration (Barker, 2008; Behari, 2010; Li et al., 2018). CTNNB1 mutations, which are observed more frequently in male liver cancer patients (Xia et al., 2006; Li et al., 2018), induce high β-catenin activity leading to liver cancer (Rebouissou et al., 2016).

IL6 is overexpressed in almost all types of cancer; its increased expression indicates a strong correlation between inflammation and cancer (Kumari et al., 2016). IL6 expression is inhibited by estrogen in Kupffer cells in the liver, which may explain the lower incidence of liver cancer in women than men (Naugler et al., 2007; Liu and Liu, 2014). In the diethylnitrosamine-induced animal model of HCC, higher production of IL6 was reported in males than in females (Naugler et al., 2007). Our results showed that the higher expression levels of CTNNB1 and IL6, the lower the OS probability, and the difference in female liver cancer patients were all significant (Fig. 1A, 1B). It would be worthwhile to examine whether female liver cancer patients with high IL6 levels have low estrogen levels.

The Rho family is involved in cellular proliferation and metastasis in cancer via reorganization of the actin cytoskeleton and regulation of related signaling (Heasman and Ridley, 2008; De Rienzo et al., 2016). In male malignant pleural mesothelioma patients, expression of RHOA was significantly higher in non-epithelioid tumors, and was associated with a significant reduction in survival (De Rienzo et al., 2016).
this study, high RHOA expression was associated with low OS probability in both male and female liver cancer patients. When the survival period was extended to 80 months or longer, the difference in OS probability between the RHOA-high and RHOA-low expression groups was markedly greater in male than in female liver cancer patients, indicating a sex-biased effect of this gene (Fig. 1D).

The protein GLIPR1 has been shown to act as a tumor suppressor in thyroid and prostate cancers. Thyroid cancer occurs more frequently in women, but is more aggressive in men (Rahbari et al., 2010). In thyroid cancer, testosterone promotes cancer progression by reducing GLIPR1 expression and cancer immune mechanisms (Zhang et al., 2015). In prostate cancer, GLIPR1 inhibits cancer development by destructing cytosolic β-catenin and c-Myc (Li et al., 2011). It would be worthwhile to further investigate the interaction between GLIPR1 and β-catenin. In glioma, GLIPR1 functions as an oncoprotein (Murphy et al., 1995; Ren et al., 2004; Awasthi et al., 2013). The role of GLIPR1 in liver cancer has not yet been determined. This study shows a sex-biased correlation between GLIPR1 expression and OS in liver cancer patients: a negative correlation in males and a positive correlation in females (Fig. 1C).

In this study, correlations between sex-biased OS probability and the levels of expression of the four genes develop into a meaningful result. It can be expected that this is due to the influence of sex hormones, especially estrogen. Because the action of the female sex hormone estrogen is the main reason why liver cancer is superior to male (Bray et al., 2018; Jung et al., 2019). Of the four sex-biased genes from liver cancer through literature search and database analysis, CTNNB1 and IL6 have been reported to have estrogen correlation.

Crosstalk between Wnt signaling pathway, the representative signaling mechanisms related to CTNNB1, and estrogen

Fig. 2. Sex difference of the marker genes in predicting the clinical outcomes of liver cancer patients. Group 1 (g1), liver cancer patients with high expression of CTNNB1, IL6, and RHOA and low expression of GLIPR1; Group 2 (g2), the other patients. (A) In all liver cancer patients; (B) In female and (C) male liver cancer patients.
signaling pathway increase cell growth by inducing the transcription of cyclin D1 gene, CCND1, and stabilization of cyclin D1 protein (Kouzmenko et al., 2004; Mulligan et al., 2017). As mentioned earlier in the introduction, the previous studies showed that the risk of liver cancer is lower in female by suppressing IL6 secretion from Kupffer cells due to the estrogen signal (Naugler et al., 2007; Liu and Liu, 2014). There are also some studies showing that estrogen decreases the activity of the transcription factors nuclear factor κB (NF-κB) and CCAAT/Enhancer Binding Protein (CEBPp), thereby reducing the promoter activity of IL6 (Stein and Yang, 1995).

There are few studies of the association between estrogen and RHOA or GLIPR1 in liver cancer. According to Saillard’s study, inhibition of estrogen-related receptor α (ERRα) in breast cancer increases the stability and activation of RhoA protein, which affects cell migration (Saillard et al., 2014). In another study, RHOA and sex hormones were found to have no significant relevance in the primary breast cancer (Bellizzi et al., 2008). There are no studies on the relationship between GLIPR1 and estrogen. In this study, the relationship between RHOA or GLIPR1 expression and sex-biased OS in liver cancer patients shows the need for further study.

SEX-BIASED MOLECULAR SIGNATURE FOR OVER-ALL SURVIVAL OF LIVER CANCER PATIENTS

In order to predict clinical outcomes of liver cancer in a sex-biased manner, four genes in this study were investigated further the possibility as a sex-biased molecular signature. To this end, these four genes grouped together and analyzed the TCGA database for overall survival of male and female liver cancer patients. The patients were stratified into two groups based on expression of the genes: group 1 (g1) had the profile CTNNB1 High, IL6High, RHOAHigh and GLIPR1Low, whereas group 2 (g2) included the other patients. For each gene, high and low expression were determined by comparison with the average expression level for each gene. As shown in Fig. 2A, KM plot analysis exhibited no significant difference in OS probability between g1 and g2 when male and female data were analyzed together. However, when data from males and females were analyzed individually, different results were obtained. In female patients (N=121), g1 exhibited worse prognostic outcomes compared to g2 (HR=2.224, p=0.010, Fig. 2B). In contrast, in male patients (N=250), there was no significant difference in OS between the two groups (HR=0.931, p=0.805, Fig. 2C). Taken together, these results suggest that high expression of CTNNB1, IL6, and RHOA and low expression of GLIPR1 may serve as a prognostic biomarker for female liver cancer patients. The sex-biased molecular signature CTNNB1High, IL6High, RHOAHigh, and GLIPR1Low proposed in this study may serve as an index for prediction and evaluation of OS in liver cancer, specifically in female liver cancer patients.

CONCLUSION

Sex/gender is an important biological variable that should be considered in all cancer research that aims to improve targeted therapies (Gabriele et al., 2016). Diseases cover both sex and gender concepts. In the present study, we demonstrated that the expression pattern of a set of genes (CTNNB1High, IL6High, RHOAHigh, and GLIPR1Low) was associated with poor OS probability in female liver cancer patients. The sex-biased molecular signature proposed in this study can be used to predict and evaluate the prognosis of liver cancer specifically in female patients. Further studies are needed to elucidate the molecular mechanisms underlying our findings.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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