Prostate Stem Cell Antigen and Pancreatic Cancer

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1 Introduction

Pancreatic cancer is a highly fatal neoplasm and the fourth leading cause of cancer death in the United States. It is usually silent until the advanced stage, where it is recognized through symptoms that result from an invasion of tumor cells into the surrounding tissues, such as abdominal pain and obstructive jaundice, or through symptoms due to metastasis to distant organs. Even with a modern health-check system, it is difficult to recognize an early-stage tumor, as the small but highly malignant tumor cannot be detected by MRI or ultrasound imaging. Therefore, a sensitive and accurate blood marker is anticipated for utilization in patient screening. Recently, several candidate molecules for the blood marker have been reported, which include prostate stem cell antigen (PSCA). In this article, the biological character and clinical utility of PSCA are reviewed in the context of pancreatic cancer.

2 Pancreatic Cancer

2.1 Clinical Character of Pancreatic Cancer

The pancreas consists of mainly three types of cells: acinar cells secreting digestive enzymes, ductal cells forming ducts which transport the digestive enzymes to the duodenum, and islet cells secreting hormones such as insulin and glucagon. More than 85% of pancreatic tumors are of ductal cell origin (Basturk et al., 2010). Therefore, ductal adenocarcinoma has actually become synonymous with pancreatic cancer. In this article, the term pancreatic cancer indicates ductal adenocarcinoma unless it is described with other particular histologic types.

Pancreatic cancer is the fourth leading cause of cancer death in the United States and 227,000 patients are estimated to die every year worldwide (See Vincent et al., 2011 for review). Early-stage pancreatic cancer is usually silent until the patients have symptoms caused by invasion of cancer cells into surrounding tissues or metastasis to distant organs. In other words, most pancreatic cancer patients with symptoms have already reached the advanced stage. Typical symptoms are abdominal or mid-back pain, obstructive jaundice and weight loss. Tumors developed in the pancreatic head would be more susceptible to early diagnosis than those in the pancreatic tail, as the former often involve the common hepatic duct or the proximal side of the pancreatic duct in early stage, resulting in the development of recognizable symptoms such as obstructive jaundice. About 25% of patients with pancreatic cancer have diabetes mellitus at diagnosis and another 40% have impaired glucose tolerance, but the cause of these abnormalities in glucose tolerance is unknown (Chari et al., 2008). New-onset diabetes in older people could suggest the development of pancreatic cancer.

Tri-phasic (i.e., arterial, late arterial, and venous phases) pancreatic-protocol CT is the best initial diagnostic test for pancreatic cancer, and optimum CT scans including 3-dimensional reconstruction have 80% accuracy for the prediction of resectability (Vincent et al., 2011). Endoscopic ultrasound is also a highly reliable procedure for diagnosing pancreatic cancer, and, at the same time, fine-needle aspiration of the pancreatic mass could be performed to obtain cytological samples, with about 80% sensitivity for diagnosis (Harewood & Wiersema, 2002).

A complete cure can be obtained only by successful resection of the entire tumor mass by pancreatic-duodenectomy; however, for patients with surgically resected ductal adenocarcinoma of the pancreatic head, actuarial 5-year overall survival is about 20–25% (Vincent et al., 2011). Adjuvant therapy is recommended for patients who undergo pancreatic resection with curative intent. The chemotherapeutic
option for pancreatic cancer includes fluorouracil and gemcitabine, and several new regimens of chemotherapy have been clinically tried in comparison with gemcitabine, which includes erlotinib, a epidermal growth factor receptor inhibitor (Cao et al., 2013). The efficacy of addition of radiation to chemotherapy is unproven and controversial.

2.2 Risk- and Genetic Susceptibility-Factors of Pancreatic Cancer

Risk factors of pancreatic cancer include smoking, family history of chronic pancreatitis, advancing age, male gender, diabetes mellitus, obesity, non-O blood group and occupational exposure to certain chemicals (Vincent et al., 2011). Among the factors, cigarette smoking and family history are important. About 7–10% of pancreatic cancer patients have a family history (Petersen et al., 2006), and the cancer is defined as familial pancreatic cancer if it occurs in families in which a pair of first-degree relatives have been diagnosed with a pancreatic tumor. First-degree relatives of individuals with familial pancreatic cancer are associated with a 9-fold increased risk of pancreatic cancer, and patients with familial pancreatic cancer have more precancerous lesions than those with sporadic pancreatic cancer (Klein et al., 2004; Brune et al., 2010). The risk of pancreatic cancer is also modestly increased in the case of sporadic pancreatic cancer. Other than that described above, alcohol consumption may also be a risk factor. One study revealed that alcohol consumption, specifically liquor consumption of 3 or more drinks per day, increases pancreatic cancer mortality independent of smoking (Gapstur et al., 2011). Red meat intake, particularly meat cooked at high temperatures and associated with mutagens, was suggested to play a role in pancreatic cancer development (Stolzenberg-Solomon et al., 2007). Intriguingly, recent meta-analyses showed an association between infection with Helicobacter pylori and the development of pancreatic cancer (Pooled adjusted odds ratio 1.38; 95% Confidence interval: 1.08-1.75; P value=0.009, Trikudanathan et al., 2011).

Recently, several genomewide association studies (GWAS) using a single nucleotide polymorphism (SNP) as a genetic marker were conducted on a germline DNA sample set of a case-control cohort, to capture genes related to pancreatic cancer susceptibility. One study revealed an association between a SNP in the first intron of the ABO blood group gene and pancreatic cancer (Amundadottir et al., 2009). The ABO gene encodes a glycosyltransferase that catalyzes the transfer of carbohydrates to the H antigen, forming the antigenic structure of the ABO blood groups. Although the reason for the association has not been uncovered, the result supports earlier epidemiologic evidence suggesting that people with blood group O may have a lower risk of pancreatic cancer than those with groups A or B. Another GWAS identified eight SNPs that map to three loci on chromosomes 13q22.1, 1q32.1 and 5p15.33 (Petersen et al., 2010). Two correlated SNPs, rs9543325 and rs9564966, map to a 600-kb nongenic region between two genes, kruppel-like transcription factor-5 (KLF5) and KLF12, on chromosome 13q22.1. Five SNPs on 1q32.1 map to the NR5A2 gene encoding nuclear receptor subfamily 5, group A, member 2. The NR5A2 protein is a nuclear receptor of the fushi tarazu (Ftz-F1) subfamily which is predominantly expressed in the exocrine gland of the pancreas, the liver, intestine and ovaries in an adult. It interacts with β-catenin to activate expression of cell-cycle genes (Botrugno et al., 2004), which seems to be the reason for the correlation between the SNPs and pancreatic cancer. The one remaining SNP maps to 5p15.33, residing in intron 13 of CLPTMIL (encoding cleft lip and palate transmembrane 1-like). CLPTMIL is part of the CLPTMIL-TERT locus that includes TERT (encoding telomerase reverse transcriptase), which is only 23 kb distant from the gene. CLPTMIL is up-regulated in cisplatin-resistant cell lines and may play a role in apoptosis (Yamamoto et al., 2001), while TERT encodes the catalytic subunit of telomerase, which is essential for maintaining telomere ends. This locus has been identified in a GWAS of a number of different cancers, including brain tumors, lung cancer, basal cell carcinoma and melanoma (Wang et al., 2008;
Pancreatic intraepithelial neoplasia (PanIN) is a major precursor lesion for pancreatic ductal carcinoma and the oncogenic molecular events occurring in its development have been well elucidated (Feldmann & Maitra, 2010). In accordance with the progression of the degree of histological atypism, PanIN is classified into three stages: PanIN-1 (with mild atypia), PanIN-2 (with moderate atypia) and PanIN-3 (carcinoma-in-situ), with PanIN-1 being further divided into PanIN-1A (flat lesion) and -1B (papillary lesion). The progression of PanIN is highly associated with a progressive accumulation of genetic aberration (Figure 1, Maitra et al., 2003). Concordant with its pathogenesis, basically, pancreatic cancer develops in older people, and more than half of the population above the age of 65 has PanIN (Andea et al., 2013).

The genetic events occur in a well-characterized sequence rather than in a random manner (Figure 1). In the early stage, KRAS activation resulting from a mutational event is important, as it was reported that an activating KRAS mutation resulted in the development of PanIN in transgenic mice (Hingorani et al., 2003). Indeed, in humans, oncogenic mutations in KRAS were found in 36% of PanIN-1A, 44% of PanIN-1B and 87% of PanIN 2/3 lesions (Feldmann et al., 2007). The KRAS activation initiates succeeding oncogenic signals: Raf/MEK/ERK pathway, PI3K/Akt pathway and Ral-GEF pathway. A recent study revealed that WNK2 gene is down-regulated in PanIN via hypermethylation of its promoter (Dutrue et al., 2013). As the gene encoding protein kinase is known to negatively regulate an activation of the MEK1/ERK pathway in cancer cells, its down-regulation may enhance activation of the pathway by KRAS. Loss of the CDKN2A/p16 gene is observed in 30% of PanIN-1, 55% of PanIN-2 and 71% of PanIN-3 (Wilentz et al., 1998). The gene encodes a cell-cycle checkpoint protein and its inactivation causes dysfunction in cell-cycle arrest in G2-S transition. Loss of TP53 function was observed in 50–75% of pancreatic cancer (Feldmann et al., 2007). Nuclear accumulation of TP53 protein reflects the mutational status of TP53 and is observed in PanIN-3 (Maitra et al., 2003). As TP53 is involved in cell-cycle regulation and apoptosis induction, its inactivation is prone to promote carcinogenesis. SMAD4 is one of the downstream molecules in TGF-beta cell growth inhibition signaling. SMAD4 expression is intact in PanIN-1 and PanIN-2 lesions, but loss of SMAD4 expression is observed in 31% of PanIN-3 lesions (Wilentz et al., 2000). BRCA2 is also a tumor-suppressor, which is inactivated in the advanced stage (Goggins et al., 2000). A genome-wide copy number analysis on individuals with a family history of pancreatic cancer revealed that somatic chromosomal copy number changes were identifiable in only 8 lesions of the 38 PanIN or intraductal papillary mucinous neoplasms (IPMN), and that only two precursor lesions had more than one somatic copy number alteration, while the overwhelming majority (~95%) of PanINs harbored KRAS mutations, suggesting that there is no one tumor suppressor gene locus consistently involved in initiating familial pancreatic neoplasia (Hong et al., 2012). Another genome-wide copy number analysis on pancreatic ductal adenocarcinoma disclosed frequent gains of 1q, 2, 3, 5, 7p, 8q, 11, 14q and 17q (> or =78% of cases), and losses of 1p, 3p, 6, 9p, 13q, 14q, 17p and 18q (> or =44%), and the SKAP2/SCAP2 gene (7p15.2), which belongs to the Src family kinases, was most frequently (63%)
amplified with a significant correlation between its DNA copy number and mRNA expression level (Harada et al., 2008).

Recent exome sequencing revealed aberrations in axon guidance pathway genes (Biankin et al., 2012). Notably, inactivation of SLIT/ROBO signaling was observed 20% of pancreatic ductal adenocarcinoma. It is suggested that SLIT/ROBO signaling modulates MET and WNT signaling activity through CDC42 and β-catenin, respectively. MET, a well-known proto-oncogene encoding a hepatocyte growth factor receptor with tyrosine-kinase activity, was known to have a role in a population of self-renewing cancer stem cells of pancreatic cancer (Li et al., 2011), and it was recently reported that canonical Wnt signaling is required for pancreatic carcinogenesis (Zhang et al., 2013a). Consequently, the loss of SLIT/ROBO signaling may promote pancreatic carcinogenesis. The exome sequencing was also performed by two other groups (Jones et al., 2008; Murphy et al., 2013). As a result of the 3 studies, 4 genes were listed as frequently mutated genes common to the 3 studies: KRAS, TP53, SMAD4 and ATM.

MicroRNAs (miRNAs) are 18- to 22-nucleotide-long, noncoding RNAs. Accumulating evidence suggests that they are involved in an important biological process via regulation of gene expression. Three miRNAs, miR-21, miR-155 and miR-200, are frequently expressed in pancreatic cancer tissues and are supposed to have a significant role in carcinogenesis (Tang et al., 2013). For example, miR-155 represses expression of TP53INP1 (P53 inducible nuclear protein) and prevents pancreatic cancer cells from apoptosis. The field of noncoding RNA is still progressing and will provide important knowledge about pancreatic carcinogenesis.

![Figure 1: Development of pancreatic intraepithelial neoplasia (PanIN) and associated genetic changes. According to its histological atypism, PanIN is classified into three stages: PanIN-1 to -3. Aberrant expression of PSCA is started even in the early stage of carcinogenesis, in which KRAS activation is also identified, suggesting its contribution to carcinogenesis. (Adapted by permission from Macmillan Publishers: Modern Pathology (Maitra et al., 2003), copyright 2003).](image-url)
2.4 Pancreatic Neuroendocrine Tumor

Pancreatic neuroendocrine tumor (PanNET) is relatively rare and said to be 1-2% of all pancreatic neoplasm (Asa, 2011). However, because of its clinically asymptomatic feature, it was reported that one of every five incidentally diagnosed pancreatic tumors is a PanNET, and up to 40% of all PanNETs are incidentally diagnosed, suggesting there may be more cases than the number diagnosed at hospitals (Zhang et al., 2013b). PanNETs are of islet cell origin and known to release hormones such as insulin and glucagon, but 85% of them are non-functional (Franko et al., 2010).

The genetic events that occur in PanNET carcinogenesis are totally different from those of ductal adenocarcinoma, and KRAS mutation is not actually observed. In brief, important in the carcinogenesis are gene alternations that result in activation of PI3K (phosphoinositide-3-kinase)/Akt (v-akt murine thymoma viral oncogene) signaling and mTOR (mechanistic target of rapamycin serine/threonine kinase) pathway, and endothelial-independent islet cell survival (See Zhang et al., 2013b for review). It was demonstrated that Akt activation was observed in many PanNET and that mutation in the genes of two inhibitory molecules for PI3K/Akt signaling, PTEN and TSC (tubrous sclerosis complex) was detected in 7.4 and 8.8%, respectively, of the PanNET cases (Ghayouri et al., 2010; Krausch et al., 2011; Krymskaya & Goncharova, 2009). Menin encoded by the MEN1 gene promotes expression of 2 genes encoding cell cycle inhibitors, CDKN1B and CDKN2C, in the downstream of the PI3K/Akt signaling, and mutation in the MEN1 was found in 44% of sporadic PanNETs (Jiao et al., 2011). The mTOR pathway promotes cell survival, proliferation and motility and is activated in PanNETs (Zhou et al., 2011).

3 Prostate Stem Cell Antigen

3.1 Contextual Expression of PSCA

Prostate stem cell antigen (PSCA) is a glycosylphosphatidylinositol (GPI)-anchored cell surface protein belonging to the Thy-1/Ly-6 family (Figure 2). It has123 amino acids and consists of an amino-terminal signal sequence, a carboxy-terminal guanosyl-phosphatidylinositol-anchoring sequence, and multiple N-glycosylation sites (Bahrenberg et al., 2000). PSCA protein is detected between 10 and15 kDa molecular-weight markers in western blot analyses; however, PSCA protein with a larger size was also reported (Reiter et al., 1998; Tanikawa et al., 2012). Several antibodies were reported to be used in immunohistochemical analyses of PSCA (Gu et al., 2000, Sakamoto et al., 2008, Geiger et al., 2011). Its expression in humans has been reported in the epithelia of prostate, urinary bladder, kidney, skin, esophagus, stomach, gallbladder and placenta (Reiter et al., 1998; Bahrenberg et al., 2000; Gu et al., 2000; de Nooij-van Dalen et al., 2003; Ono et al., 2012a). PSCA expression was also observed in the telencephalon and peripheral ganglia of the nervous system in mice (Hruska et al., 2009). Recently, its expression in islet cells of the normal pancreas was demonstrated by our groups (Ono et al., 2012b), and will be described later in this article. Our knowledge about PSCA-expression sites in the human body is limited, and other cryptic expression sites might be exhibited in the future.

Initially, PSCA was identified as a gene over-expressed in prostate cancer (Reiter et al., 1998) and succeeding investigations revealed it is also up-regulated in urinary bladder cancer, renal cell carcinoma, pancreatic cancer, hydatidiform mole, ovarian mucinous tumor, glioma and lung cancer (Amara et al., 2001; Elsamman et al., 2006; Argani et al., 2001; Feng et al., 2008; Cao et al., 2005; Geiger et al., 2011; Kawaguchi et al., 2010); however, it is down-regulated in esophageal, gastric and gallbladder cancers
Figure 2: PSCA is a glycosylphosphatidylinositol (GPI)-anchored cell surface protein. It is thought to localize to lipid raft by its GPI moiety and be involved in subcellular signal transduction. However, molecules interacted with PSCA protein are yet to be identified.

(Bahrenberg et al., 2000; Sakamoto et al., 2008; Ono et al., 2012a) (Table 1, Figures 3-5). It was identified as a gastric-cancer susceptibility gene by GWAS, for the first time, on the Japanese population (Sakamoto et al., 2008), and the association between the gene and gastric cancer was replicated in other ethnic populations including Caucasian (Saeki et al., 2013). It was also found as a bladder-cancer susceptibility gene by GWAS (Wu et al., 2009). Intriguingly, a recent GWAS revealed an association of PSCA with duodenal ulcer, although it is not expressed in the duodenum (Tanikawa et al., 2012).

<table>
<thead>
<tr>
<th>Up-regulation</th>
<th>Down-regulation</th>
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<tr>
<td>Glioma (Geiger et al., 2011)</td>
<td>Esophageal squamous cancer (Bahrenberg et al., 2000)</td>
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<tr>
<td>Lung non-small cell carcinoma (Kawaguchi et al., 2010)</td>
<td>Gastric adenocarcinoma (Sakamoto et al., 2008)</td>
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<td>Pancreatic ductal carcinoma (Argani et al., 2001)</td>
<td>Gallbladder adenocarcinoma (Ono et al., 2012a)</td>
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<tr>
<td>Prostate carcinoma (Reiter et al., 1998)</td>
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<tr>
<td>Urinary bladder carcinoma (Amara et al., 2001)</td>
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<tr>
<td>Renal cell carcinoma (Elamman et al., 2006)</td>
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<td>Ovarian mucinous tumor (Cao et al., 2005)</td>
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<td>Hydatidiform mole (Feng et al., 2008)</td>
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Table 1: Expression status of PSCA in cancer.
Figure 3: Immunohistochemistry showing PSCA expression (brown) in normal prostate gland (left panel) and its upregulation in prostate cancer (right). Immunohistochemistry was conducted using anti-PSCA antibody produced by our group and anti-mouse IgG antibody labeled with horseradish peroxidase. The localization of the antibodies was visualized using VECTASTAIN Universal Quick Kit (Vector Laboratories, Burlingame, CA, USA). Bar, 100µm.

Figure 4: PSCA is expressed in the epithelium of gastric mucosa. (a) The gastric mucosa consists of 4 regions: base, neck, isthmus and pit, and stem cells at the base region differentiate to mature cells in gastric mucosa. PSCA (blue) is expressed in the middle portion of gastric mucosa, which harbors a pre-pit cell, a transit amplifying cell differentiating to pit cell. (b) PSCA is down-regulated in diffuse-type gastric cancer. Arrowheads indicate PSCA expression in normal gastric epithelial cells. Immunohistochemistry using anti-PSCA antibody produced by our group, double-stained with anti-PCNA (proliferating cell nuclear antigen) antibody (red). See Sakamoto et al., 2008 for procedure details. Bar, 100µm.

As described above, PSCA shows a contextual expression pattern in normal and cancer tissues, but the regulatory mechanism of PSCA expression is almost unknown. It was reported that, in bladder carcinoma cell line RT112, PSCA expression was up-regulated by contact of the cells to a culture dish surface and resulted in an aggregation of cells, and also by phorbol ester, indicating that its expression is regulated by mechanisms related to the adhesion of epithelial cells (Bahrenberg et al., 2001).
Figure 5: PSCA is down-regulated in gallbladder cancer. PSCA expression is observed in normal gallbladder epithelium (a) and it is down-regulated in well (b), moderately (c) and poorly (d) differentiated gallbladder carcinoma. Immunohistochemical double-staining for PSCA (blue) and PCNA (proliferating cell nuclear antigen, red). See Ono et al., 2012a for details of the study, and Sakamoto et al., 2008 for details of procedure.

Androgen seems to be involved in PSCA regulation, at least in the prostate epithelium, as an androgen responsive element was identified in its promoter region (Jain et al., 2002) and transgenic mice introduced with PSCA promoter-driven GFP constructs exhibited GFP expression which was influenced by puberty, castration and androgen restoration (Watabe et al., 2002). In humans, complete androgen ablation with bicalutamide and goserelin acetate decreased the number of PSCA-expressing cancer cells in 2/3 cases of prostate cancer (Zhigang & Wenlu, 2005). On the other hand, androgen-independent regulation is also suggested. 15-Lipooxygenase-2 (15-LOX2) is a human-specific, non-hem and iron-containing enzyme that metabolizes arachidonic acid to 15(S)-hydroxy-eicosatetraenoic acid, and transgenic expression of 15-LOX2 in mouse prostate resulted in age-dependent prostatic hyperplasia associated with expression of several stem or progenitor cell molecules, including Sca-1 and Psca (Suraneni et al., 2010).

Intriguingly, it was also reported that PSCA is down-regulated in telomerase-transduced urothelial cells (Chapman et al., 2008). DNA methylation was implicated as a mechanism of the down-regulation in gastric and gallbladder cancers (Ono et al., 2012a).

3.2 Function of PSCA

Although the GPI-moiety is a common feature, the members of the GPI-anchored proteins have significant diversity in their structure and function (Chatterjee & Mayor, 2001) (Table 2). The GPI-anchored proteins lack a transmembrane domain and are thought to locate in lipid rafts, a special microdomain, enriched in specific proteins and lipids, of the surface of the outer cell membrane. In the lipid raft, the
GPI-moiety may have the capacity to transduce some signals across the cell membrane, and in fact, several studies demonstrated that cross-linking of the GPI-anchored proteins by antibodies elicit the signal transduction. A mouse monoclonal anti-PSCA antibody 1G8 inhibited tumor growth and metastasis, and prolonged the survival of mice that had been inoculated with human prostate cancer cell lines as xenografts, which is probably through inducing caspase-independent cell death of the cancer cells by cross-linking the proteins (Gu et al., 2005). On the other hand, in chicken brain, Psca prevents a subpopulation of choroid cells from cell death by modulating a signaling pathway involving α7-containing nicotinic acetylcholine receptors (Hruska et al., 2009). In addition, PSCA showed cell growth inhibition activity for gastric and gallbladder cancer cell lines, instead of cell death induction (Sakamoto et al., 2008; Ono et al., 2012a). In spite of its designation as stem cell antigen for its structural similarity to stem cell antigen 2 (SCA-2) (Reiter et al., 1998), PSCA is expressed mainly in differentiating cells rather than in stem cells, which was revealed in a study on prostate and gastric epithelial cells (Tran et al., 2002; Sakamoto et al., 2008); however, PSCA expression can also be observed in mature or differentiated cells in gallbladder epithelium and pancreatic islet (Ono et al., 2012a; Ono et al., 2012b). Although PSCA is expressed in several tissues in the human body, a detailed expression pattern in the context of cell or tissue differentiation is not well elucidated in tissues other than prostate epithelium. It could be supposed that the pattern also depends on tissue type.

<table>
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<tr>
<th>Function</th>
<th>GPI-proteins</th>
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<tr>
<td><strong>Enzymes</strong></td>
<td>Acetylcholinesterase (Roberts &amp; Rosenberry, 1985)</td>
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<td></td>
<td>Alkaline phosphatase (Low &amp; Finean, 1977)</td>
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<td></td>
<td>5′-nucleotidase (Zekri et al., 1989)</td>
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<tr>
<td><strong>Adhesion molecules</strong></td>
<td>Neural cell adhesion molecule (Powell et al., 1991)</td>
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<td></td>
<td>Contactin (Reid et al., 1994)</td>
</tr>
<tr>
<td></td>
<td>Neurotrimin (Struyk et al., 1995)</td>
</tr>
<tr>
<td><strong>Receptors</strong></td>
<td>Folate receptor 1 (Rijnboutt et al., 1996)</td>
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<td></td>
<td>GDNF Family Receptor Alpha 1 (Treonor et al., 1996)</td>
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<td></td>
<td>Plasminogen Activator, Urokinase Receptor (Casey et al., 1994)</td>
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<tr>
<td><strong>Surface antigens</strong></td>
<td>Thy-1 cell surface antigen (Tse et al., 1985)</td>
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<tr>
<td></td>
<td>CD48 (Korinek et al., 1999)</td>
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<td></td>
<td>Glypican 2 (Stipp et al., 1994)</td>
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**Table 2:** Functional diversity of GPI-anchored protein

Moreover, complicated as it is to understand its physiological significance, PSCA function seems to be contextual. The expression status of PSCA in cancer cells is dependent on the epithelium of their origin; up-regulated in prostate and urinary bladder cancers but down-regulated in esophageal and gastric cancers. This suggests that it could be oncogenic for some epithelial cells and also be a tumor suppressor for others. This contradiction may be related to the integrity of the cell polarity. One hypothesis is that PSCA basically has a role in regulation of cell proliferation and/or differentiation, which contributes to preventing a malignant transformation of the cells; but once the cell polarity is destroyed, it may act in tumor progression dependent on cell type. Mucin1 (*MUC1*), encoding a mucous protein in gastric mucous secreted by pit cells, is a good example. *MUC1* is a membrane-bound protein (Bafna et al., 2010). After
being translated, a single MUC1 peptide is cleaved to N-terminal and C-terminal subunits, designated as MUC1-N and MUC1-C, respectively, but both the subunits are localized together to the cell membrane in the apical side of the epithelial cells. MUC1-C has a transmembrane domain and a cytoplasmic tail (CT) which is involved in subcellular signal transduction. On the other hand, MUC1-N present on the cell surface has multiple glycosylation sites and is thought to act in protection against many types of insults, after the front layer of defense by the secretary mucins in mucus. In a normal epithelium, MUC1 protein is localized to the apical side of the cells, which restricts interaction between MUC1 and other membrane proteins involved in signal transduction, and MUC1 is protective against environmental insults potentially leading to tumorigenesis. However, after significant epithelial damage, the cells lose their polarity and the membrane proteins change their distribution, which enables the membrane proteins to interact with MUC1 and finally results in eliciting MUC1’s signaling promotional for cancer cell growth (Kufe, 2009).

On the other hand, it is worthwhile to consider the PSCA function in the immune system, as it is a membrane protein. One study revealed that down-regulation of PSCA using shRNA caused reduced cell proliferation of bladder cancer cell xenografts harbored in mice, and that the PSCA down-regulation in the bladder cancer cells resulted in up-regulation of genes involved in the interferon α/β signaling pathway as well as the Interleukin 1 signaling pathway in vivo, suggesting PSCA expressed in cancer cells counteracts a natural immune response through blocking the IFNa/β receptor (Marra et al., 2010). PSCA may have a role in immune reaction in carcinogenesis such as immune escape and attack, in the context of the state and/or type of the cancer cells.

In conclusion, it is very likely that PSCA function is related to several subcellular signalings in the cells, depending on both the origin of the cells and their condition, i.e., normal or malignant. Psca knockout mice have been generated but these showed no abnormal phenotype except that the mice were susceptible to metastasis when implanted with cancer cell xenografts (Moore et al., 2008).

4 PSCA and Pancreatic Cancer

4.1 PSCA is Expressed in Islet Cells but Not in Ductal Cells in Normal Pancreas

Recently, PSCA expression was observed in islet cells of the pancreas (Ono et al., 2012b). Our immunohistochemical study revealed diffuse expression of PSCA in the islets (Figure 6). In our observation, acinar or ductal cells showed no PSCA expression.

This islet-restricted expression suggests some important role of PSCA in endocrine cells, which remains to be elucidated. Intriguingly, double staining with islet markers demonstrated that PSCA is expressed in all cell types in the islet: α (glucagon), β (insulin), δ (somatostatin) and PP (pancreatic polypeptide) cells (Figure 7). The expression common to these endocrine cells may suggest that PSCA has a role related to hormonal secretion, though a secretion-related function of PSCA has not been reported. It seems that PSCA itself has functional diversity dependent on cell type. The islet expression gives insight into the unrevealed function of PSCA. As mentioned above, PSCA is diffusely expressed in mature gallbladder epithelium, which consists of one layer of simple columner cells and has a simple function, i.e., absorbing fluid and electrolytes to condense bile, suggesting PSCA may have a simple function in some epithelium rather than a complex function related to cell proliferation or differentiation.
Figure 6: PSCA is expressed in islet cells but not in acinar or ductal cells. Immunohistochemistry using anti-PSCA (blue) and anti-PCNA antibodies (red) shows diffuse PSCA signals in the islets suggesting it is expressed in multi-lineage of the islet cells. The right panel is a magnification of the islet demarcated in the left panel. (Adapted from Ono et al., 2012b).

Figure 7: Fluorescent immunohistochemistry of PSCA (red) double-stained with islet cell markers (blue, glucagon for α cells, insulin for β, somatostatin for δ, pancreatic polypeptide for PP) exhibits PSCA expression in all the islet cell lineages (Arrows indicate representative staining of each type of cells). The β cells are numerous and situated in the central regions of the islets. The α cells are generally arranged around the periphery. The δ and PP cells are less numerous and do not display an obvious pattern of the arrangement (Klimstra et al., 2007). Bar, 25µm. (Adapted from Ono et al., 2012b).
The pancreatic expression was confirmed by detecting *PSCA* transcripts in RNA from pancreatic tissues. Intriguingly, in conducting an RNA ligase-mediated rapid amplification of 5’ cDNA end procedure in order to elucidate the promoter region used for the pancreatic expression, we also detected a variant of the *PSCA* transcript, that encodes no protein (variant 2 in Figure 8a) (Ono 2012b). We extended the variant study to other tissues and their neoplastic lesions, which revealed that the non-coding variant is also expressed in stomach, gallbladder and their cancer cell lines (Figure 8b).

![Diagram](image)

**Figure 8**: Expression of non-coding *PSCA* transcripts in pancreas, stomach, gallbladder and their cancer cell lines. (a) A schematic representation of the structure of the authentic (variant 1) and non-coding variant (variant 2). (b) Quantitative PCR detected variant 1 and 2 transcripts in gastric, gallbladder and pancreatic tissues and their cancer cell lines. TSS, transcription starting site. ARE, androgen responsive element. Quantitative RT-PCR was performed with gene expression assay using SYBR Premix Ex Taq II (Takara Bio Inc, Shiga, Japan), conducted in 40 cycles under a condition of 2 steps of temperature: 95°C for 5sec and 60°C for 30sec, by the ABI PRISM 7900HT Sequence Detection System. The relative transcript level was calculated using the Ct value of *GAPDH* transcript as reference. (Adapted from Ono *et al.*, 2012b).
The expression of the non-coding variant could have some important physiological function including moderation of PSCA expression. For example, the 3' untranslated region (UTR) of variant 2, which is shared with variant 1, may act as a decoy in recognition by microRNA or other molecules. Intriguingly, variant 2 is a dominant PSCA transcript in some pancreatic and gallbladder cancer cell lines (Ono et al., 2012b). The transcription start site (TSS) of variant 2 locates about 10-kb upstream to that of variant 1, which is even upstream to the androgen responsive element located about 3-kb upstream to the variant 1 TSS. This distance between the two TSS’s suggests that transcription of the 2 variants is regulated by distinct promoters. It may be possible to hypothesize that variant 2 expressions may contribute to carcinogenesis in some tissues, like stomach and gallbladder, in which PSCA has a role in tumor suppression.

4.2 PSCA is Aberrantly Expressed in Pancreatic Cancer

PSCA expression in pancreatic cancer was reported for the first time by Argani et al (Argani et al., 2001). They compared SAGE (serial analysis of gene expression) libraries derived from pancreatic adenocarcinoma to those derived from non-neoplastic pancreatic specimens and reported that PSCA was expressed in four of the six pancreatic cancer SAGE libraries but not in the libraries from normal pancreatic ductal cells. They also detected PSCA over-expression in 60% of primary pancreatic adenocarcinoma by immunohistochemistry but not in 59 of 60 specimens from the adjacent non-neoplastic pancreas (Figure 9). Hypomethylation in the 5' CpGs of the PSCA gene was suggested for the causal of the over-expression of PSCA in pancreatic cancer tissues and cell lines (Sato et al., 2003).

![Figure 9: PSCA is up-regulated in pancreatic ductal carcinoma. PSCA expression is observed in cancer cells (arrows). Adjacent normal pancreatic ductal cells are weakly stained, probably as background signall (demarcated by arrowheads). Immunohistochemical double-stain for PSCA (blue) and PCNA (red). See Sakamoto et al., 2008 for details of procedure. Bar, 100µm.](image)
4.3 PSCA is a Useful Marker in the Diagnosis of Pancreatic Cancer

There have been several reports on the utility of PSCA in diagnostic procedures for detecting pancreatic cancer cells. It was reported that the copy number of PSCA transcripts was significantly higher in the blood of patients with metastasis of pancreatic cancer (n=9) than in the blood of patients with benign pancreatic tissues (Grubbs et al., 2006), and also that PSCA expression was present in the blood of 22 out of 47 (46.8%) patients with malignant tumors (11 pancreatic carcinoma, 8 gastric cancer, 15 colorectal carcinoma and 13 miscellaneous tumors), particularly in 7 out of 11 (63.6%) patients with pancreatic cancer (Lukyanchuk et al., 2003). Tanaka et al. examined the plasma level of IgG antibodies reactive to 57 peptides encoded by PSCA and reported that the levels of IgGs reactive to each of the 10 different peptides were significantly higher in the plasma of pancreatic cancer patients than in that of patients with a non-neoplastic pancreatic lesion (Tanaka et al., 2007). The 3 reports suggest that PSCA is a valuable biomarker in the blood for the diagnosis of pancreatic cancer. Fukushima et al. reported its usefulness in pathological examination and RT-PCR analyses to discriminate mucinous cystic neoplasms and non-neoplastic pseudocysts located in the pancreas (Fukushima et al., 2004). PSCA was also reported as a useful marker in the cytological examination of pancreatic specimens obtained by fine-needle aspiration (sensitivity 84%, specificity 91%) (McCarthy et al., 2003), and PSCA showed different expression pattern \((P \leq 0.001)\), between benign and malignant pancreatic tissues, in RT-PCR analyses on pancreatic juice collected by intraoperative aspiration of the main pancreatic duct (Oliveira-Cunha et al., 2011).

However, recently, the usefulness of PSCA in the diagnosis of pancreatic cancer was re-evaluated. The antibodies against five proteins, prostate stem cell antigen, fascin, 14-3-3 sigma, mesothelin and S100P (S100 calcium binding protein P), were studied for their utilization in immunohistochemistry on paraffin sections from cellblocks of the samples obtained by fine-needle aspiration, and as a result, S100P was revealed as the best diagnostic character showing 90% sensitivity and 67% specificity, while PSCA and 14-3-3 showed high sensitivity but zero specificity. Moreover, S100P correctly predicted six of seven cancers (Dim et al., 2011). In another study which evaluated pancreatic tumor markers including PSCA, it was concluded that pVHL (von Hippel-Lindau tumor suppressor), maspin, S100P, and IMP-3 (insulin-like growth factor II mRNA-binding protein 3) constitute the best diagnostic panel of immunomarkers in both surgical and fine-needle aspiration specimens (Liu et al., 2012). In this study, it was reported that strong background staining was frequently seen with PSCA. Thus, the specificity of the diagnosis by immunohistochemistry may depend on the quality of the antibodies. In conclusion, it seems that, although the result of immunohistochemistry-based procedures could be influenced by the quality of the antibody, RT-PCR-based examination is reliable in detecting pancreatic cancer cells in the tissue, blood and pancreatic juice, which may help early detection of pancreatic cancer in the human body.

4.4 PSCA is a Novel Target Molecule in the Treatment of Pancreatic Cancer

The effect of anti-PSCA antibodies has already been reported for pancreatic cancer xenografts in mice (Wente et al., 2005). The anti-PSCA antibody 1G8, whose tumor-growth suppression effect was demonstrated on a prostate cancer xenograft model, was intraperitoneally applied to mice subcutaneously injected with Capan-1 pancreatic cancer cells, which showed its suppressive effect on tumor formation and tumor growth.

Moreover, the combination of AGS-1C4D4, a fully human monoclonal antibody to PSCA, and gemcitabine was studied in a randomized, phase II trial with 196 patients of metastatic pancreatic cancer (Wolpin et al., 2013). In this study, the 6-month survival rate (SR) was 44.4% (95% Confidential interval
(CI), 31.9–57.5) in the gemcitabine arm and 60.9% (95% CI, 52.1–69.2) in the gemcitabine plus AGS-1C4D4 arm (P = 0.03), while the median survival was 5.5 versus 7.6 months and the response rate was 13.1% versus 21.6% in the two arms, respectively. The 6-month SR was 57.1% in the gemcitabine arm versus 79.5% in the gemcitabine plus AGS-1C4D4 arm among the PSCA-positive subgroup and 31.6% versus 46.2% among the PSCA-negative subgroup. However, among patients who received gemcitabine, the 6-month SR was higher among those with PSCA-positive tumors (57.1%) versus those with PSCA-negative tumors (31.6%), suggesting that tumor PSCA staining may also act as a prognostic marker, independent of treatment with AGS-1C4D4. The study concluded that the addition of AGS-1C4D4 to gemcitabine improved the 6-month SR among patients with previously untreated, metastatic pancreatic adenocarcinoma. This recent clinical trial suggested that additional administration of the anti-PSCA antibodies to chemotherapy reagents is a promising therapeutic strategy for metastatic pancreatic cancer. It is supposed that AGS-1C4D4 elicits an immunological response against cancer cells, as AGS-PSCA, a prototype of AGS-1C4D4, was found to induce antibody-dependent cell-mediated cytotoxicity and also to mediate complement-dependent cytotoxicity in PSCA-expressing prostate cancer xenografts in mice (Antonarakis et al., 2012).

5 Concluding Remarks and Future Prospective

PSCA is aberrantly expressed in pancreatic ductal carcinoma, suggesting its usefulness in pancreatic cancer diagnosis. As it is up-regulated in other cancers, it will be useful if it is included as one of the tumor markers in blood examination for cancer screening. For precise evaluation of the utility of PSCA in immunohistochemical diagnosis, it seems that the best quality anti-PSCA antibody should be selected, and with it, a multi-institute study should be conducted. Several lines of evidence suggest that antibodies against PSCA have a therapeutic effect on cancer. However, the molecular mechanism of the PSCA function in carcinogenesis is almost unknown. Moreover, even its function in normal cells has not been unveiled yet, although it is expressed in a variety of the organs in the human body. In pancreatic cancer and some other cancers, PSCA is likely to have an onco-promoting function. This PSCA-related oncopromoting pathway as well as PSCA itself is supposed to be a strong candidate for a therapeutic target. To develop a novel therapeutic strategy for cancer, it is important to uncover the PSCA function in carcinogenesis at the molecular level. And it should be kept in mind that PSCA is expressed in normal tissues and that it is likely to function as a tumor-suppressor in some of the tissues. To avoid any undesirable events in a PSCA-targeted cancer therapy, it is also important to explore its expression sites in the human body and its function in those tissues. The authors believe that identification of molecules that interact with the PSCA protein will be the breakthrough in PSCA investigation. We are about to use PSCA as a therapeutic target in general medical practice but we know almost nothing about its function and significance in the body.

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