

A Putative Role of Organic Anion Transporting Polypeptides (Oatps) In Cell Survival of Hormone-Dependent Breast and Prostate Cancers

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Abstract

Transporter proteins classified into the solute carrier (SLC) transporter superfamily are essential for import of nutrients for cell survival in organisms. In the last two decades, compelling evidence has accumulated that SLC transporters interact with clinically important anticancer agents and contribute to their pharmacokinetics, particularly the biopharmaceutical processes of absorption, elimination and distribution. Furthermore, many SLC transporters have been shown to be differentially upregulated in cancer cells, and this may represent an adaptive response to altered nutritional requirements. Thus, it is likely to utilize them as carrier for efficient drug delivery as well as pharmacological target to shut off the essential nutrients for cell growth of malignant tumors. This short review will introduce organic anion transporting polypeptides which recognize endo- and exogenous organic anionic compounds and recent findings about their upregulation in cancer cells. Besides, OATP-mediated sulfate conjugates of steroid hormone may contribute to cell survival and adapted growth under hormone-depleted conditions. Better understandings of pathophysiological role of OATPs likely provide key information to overcome hormone-refractory breast and prostate tumors.

Abbreviations

ADT: Androgen Deprivation Therapy; CRPC: Castration Resistant Prostate Cancer; E3S: Estrone-3-Sulfate; DHEAS: Dehydroepiandrosterone Sulfate; FDG: 2-[fluorine-18]-Fluoro-2-Deoxy-D-Glucose; OATP: Organic Anion Transporting Polypeptide; PET: Positron Emission Tomography; mTOR: Mammalian Target of Rapamycin; SLC: Solute Carrier; STS: Steroid Sulfatase.

Keywords

SLC transporters; OATP; Hormone-refractory cancer; Chemotherapy; Breast; Prostate

Introduction

Membrane transporter proteins encoded by a number of gene families may play important role in cell survival because they are essential for import of key nutrients, which is hydrophilic and impermeable to plasma membranes by itself, including glucose and amino acids. It is collectively known that many influx transporters classified into solute carrier transporter (SLC) superfamily are upregulated in malignant tumors, although their physiological relevance has to be determined.

For instances, enhanced expression of glucose transporter GLUT1/SLC2A1 in cancer cells is a molecular target for cell entry of the most common PET imaging agent in clinical use, FDG (2-[fluorine-18]-fluoro-2-deoxy-D-glucose), allowing to diagnose where malignant tumors are located [1,2]. Besides, several transporter proteins for amino acids such as leucine and glutamine have been well documented to be upregulated in many types of cancer cells, [3,4] showing their critical role in nutrient signaling to mTOR and cell growth [5]. In addition to amino acid transporter, we have shown that enhanced activity of peptide transport in cancer cells [6,7]. Thus, differential upregulation of influx transporter could be utilized not only to efficiently deliver

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their substrate drug with anti-tumor activity [3,7] or diagnostic markers [4,8,9], but also to shut off nutrient essential for cell growth; therefore, they can be promising target for a new chemotherapy. In this short review, we introduce our recent progresses regarding pathophysiological role of enhanced organic anion transporting polypeptides, OATPs, in cell proliferation of hormone-dependent breast and prostate cancers, implying pharmacological intervention of OATPs may contribute to efficient eradication of cancer cells.

OATPs

Organic Anion-Transporting Polypeptides (OATPs) are currently classified into the SLCO family consisting of 12 individual members, which have been basically characterized capable of transporting wide range of organic anionic compounds [10,11]. Human cDNAs of OATP1A2, 2B1, 1B1, 3A1 and 4A1 were originally identified in our laboratory [12] and roles of OATP2B1, 1B1 and 1A2 among those in drug pharmacokinetics and disposition have been well established by many researchers [11,12]. Members of this family generally mediate Na⁺-independent transport of amphipathic organic anion compounds and their substrates include bile salts, steroid conjugates, thyroid hormones and oligopeptides [11] as well as numerous drugs including major anticancer agents such as methotrexate [13], paclitaxel [14], SN-38 [15], and decetaxel [16]. Recent progresses about OATP family members in drug metabolism and disposition were reviewed in [17,18] with summarized list of their substrates. Nowadays, it becomes convincing that expression of OATP molecules are enhanced in many types of cancer cells; however, there is no established rationale why they are over-expressed and how they contribute to cell growth in malignant tumors.

Enhanced Expression in OATPs in Cancer Cells

OATP1B3 is one the most studied OATPs in cancer cells, and has been documented its enhanced expression in gastric, colorectal, pancreatic and breast cancer, but not hepatocellular carcinomas [13,19]. Lee et al [20] detected OATP1B3 protein expression in 75 out of 93 patient-derived colon adenocarcinomas (81%) and no immunostaining in normal samples, and more interestingly OATP1B3 exhibited antiapoptotic effect, providing a survival advantage by altering p53-dependent pathways [20], although substances transported by OATP1B3 was not identified in relation to the effect in the research. They later found that colon cancer cells express truncated form of OATP1B3 with limited transport activity because of missing the first 28 amino acids [21]. Independently, similar observation was made in cancer-type isoform of OATP1B3 mRNA that is expressed in colon and lung cancer cells. Since it was described to lack 47 amino acids at N-terminus, it may not be identical to the former one [22]. Thus, differences in expression and function may provide a clue to understand to clinical significance of OATP1B3 expression in cancer. Collectively OATP1B3 recognizes and transports major anticancer agents; therefore, such differential expression of OATP1B3 may determine their efficacy in chemotherapy, thereby resulting in providing a clinical benefit.

In addition to OATP1B3, other OATPs are also known to be highly expressed in cancer cells. A recent study with patient-derived prostate tumor specimens indicates that mRNA expression of six *SLCO* genes, including *SLCO1B3* and *2B1*, was enhanced several-fold in castration resistant prostate cancer metastases, compared to untreated prostate cancer, implying their association with prostate cancer-specific motility [23]. Previous research suggests OATP1B3 is involved in transport of testosterone [24]. Overall survival of patients with prostate cancer in response to ADT therapy is affected by genetic variants of *SLCO2B1* and *SLCO1B3*. Therefore, OATP may help prostate cancer cells to increase gonadal androgen availability [25]. Many groups including us have also implied possibility of overexpression of OATP2A1, OATP3A1, OATP4A1, OATP5A1, and OATP4C1 in breast cancer cell lines, including MCF-7 and T-47D cellsv [26-28]. OATP1A2 was shown to be upregulated in neoplastic breast tissues obtained from patients [29]. Furthermore, remarkable expression of OATP1A2 and 2B1 was reported in patient-derived human gliomas [30]. Considering the wide spectrum of substrate specificity of these transporters, these observations suggest that the transporters are one of determinants of efficacy of their substrate anti-cancer agents.

Role of OATPs in Hormone Dependent Cancer Cells

Breast cancer: It is known that steroid sulfatase (STS) activity is often detected in breast cancer cells at a considerably higher level than aromatase. STS catalyzes a hydrolysis of sulfate conjugates of steroid hormone to their unconjugated form; therefore, inactive estrone 3 sulfate (E3S) can be converted to estrone and then eventually used to generate biologically active estrogen by breast cancer cells, more likely, under estradiol-depleted conditions such as post-menopausal women. Since OATPs efficiently facilitate cell entry of hydrophilic E3S, they may contribute to breast tumor progression in cooperation with STS. This hypothesis is tested by feeding hormone-responsive breast cancer cells with E3S to determine if E3S stimulates their growth. A significant increase in MCF-7 [26] and T-47D [27] cell growth was observed. Furthermore, we have recently shown that OATP1B3 is differentially upregulated in a sub-population of MCF-7 cells, suggesting that OATP1B3 serves as E3S transporter to allow breast cancer to survive under depletion of active estrogen such as estradiol [31]. This notion is also supported by enhanced STS expression correlated with increasing grade of breast tumors in 120 clinical specimens [32]. Increased expression of OATP1A2, which can transport E3S, mediated by PXR in breast tumor tissues fed with E3S supports this as well [17,9]. These observations suggest that OATPs at least in part contributes to tumor growth by regulating hormone dependency providing an adopted cell survival of breast cancer cells. Although it remains necessary to clarify the contributions of these OATPs to tumor growth, it is conceivable that developing a potent OATPs inhibitor with high affinity kills efficiently hormone refractory breast cancer that acquired by in treatment with aromatase inhibitors such as anastrozole and letrozole.

Figure 1: Hypothesized role of OATPs in cell survival in prostate cancer cells under gonadal androgen depletion condition

Testosterone plays a role in cell growth and proliferation of prostate cancer cells and crosses the plasma membranes by simple diffusion by itself. Testosterone is converted to dihydrotestosterone (DHT) by 5 α -reductase (5 α R), and then binds to androgen receptor (AR). DHT-bound AR homodimerizes, enters nuclear and regulates gene expression so that it proceeds cell growth and releases PSA. Under the condition where testosterone is deprived of, cell entry of abundant plasma DHEAS may be facilitated by enhanced OATPs and DHEAS can be converted to DHEA, a weak androgen, by steroid sulfatase (STS), whose expression is not affected by availability of androgens. Since DHEA is converted to testosterone by multiple enzymatic reactions, handling of DHEAS by interplay of OATPs and STS may impart vulnerable prostate cancer cells an alternative source of androgens.

Prostate cancer: In human prostate cancer, gonadal androgens is critical for protein synthesis and cell survival [33]. Endocrine therapy that removes gonadal testosterone or antagonizes androgen receptors is currently a mainstream to treat prostate cancer. Although this Androgen Deprivation Therapy (ADT) is efficient, the disease may progress to the stage of Castration-Resistant Prostate Cancer (CRPC). Once CRPC is developed, it no longer responds to ADT. Since CRPC tumor progression is still considered to associate with enhanced androgen receptor (AR) function [34-36], residual androgen availability, e.g. adrenocortical androgen, may be involved in the progression. In addition to E3S, OATPs can translocate dehydroepiandrosterone sulfate (DHEAS), [11,37,38] which is a thousand fold more abundant than testosterone in human serum [39] and is basically unchanged by ADT. DHEAS is hydrolyzed to DHEA by STS [40] and then can further be converted to androstenedione, a weak androgen in prostate cancer [41], thereby resulting in stimulation of AR function (Figure 1), [42]. We have studied role of OATPs in an experimental CRPC cell culture model and recently reported OATP-mediated DHEAS is important for cell survival of prostate cancer [43]. Cell growth of AR-positive LNCaP cells was stimulated with DHEAS (Figure 2A) and the stimulation was abolished by an STS inhibitor, STX64. mRNA expression of various OATP genes were up regulated in LNCaP and 22Rv1 cells under androgen-depleted conditions [43]. Because OATP1A2 mRNA expression increased most prominently among those genes in LNCaP cells grown in androgen-depleted medium, LNCaP cells with OATP1A2 gene being silenced were established and designated KD16 and KD34. In both KD16 and 34 cells, the DHEAS-induced cell growth was not observed, compared to the control C3 and C9 cells (Figure 2B), [43]. Our results suggest that enhanced OATP1A2 expression is associated with adaptive cell growth

of prostate cancer cells under androgen-depleted conditions. Thus, OATPs including OATP1A2 may play an essential role in rescuing prostate cancer cells from shortage of androgen such as testosterone by feeding DHEAS and utilizing it as a source of androgen in interplay of STS. This provides a new rationale to complement current endocrine therapy in combination use of an efficient inhibitor for OATPs if developed.

Figure 2: DHEAS-induced cell proliferation and impact of OATP1A2-mediated DHEAS transport on proliferation of LNCaP cells.

(A) Growth of LNCaP cells was monitored by means of SRB assay for up to 7 days. LNCaP cells cultured in RPMI1640 with 10% CSS for 7 days were seeded at 8,000 cells each in the presence or absence of androgen. Each bar represents the mean \pm S.E. (n=6), and * indicates a significant difference from the control ($p < 0.05$) by Student's t-test. (B) Stimulatory effect of DHEAS on growth of OATP1A2-knockdown (open) and control cells (closed) in sulforhodamine B assay. Cells were plated at 8,000 cells in each well. The ratio of cell growth to that without DHEAS (5 μ M) was calculated for control C3 (circle) and C9 (square), and OATP1A2-knocking-down KD16 (circle) and KD34 (square) each day. Each value is the mean of 7 or 8 individual results with S.E. (n=4). These data was remade from originally reported by the authors (Ref.43).

Conclusion

There is compelling evidence that SLC transporters are upregulated in cancer cells on their demand. Among those, based on their nature, OATPs may be important players for regulating or adjusting hormone availability in hormone-dependent cancer cells. Although it is hard to exactly identify substances imported by OATPs to cancer cells, supplying sulfate conjugates of steroid hormone via OATPs allows breast and prostate cancer cells to use them as alternative source of active steroid hormones.

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