Organic Cation Transporter Preferentially Expressed in Hematopoietic Cells and Leukemias and Uses Thereof

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(54) ORGANIC CATION TRANSPORTER PREFERENTIALLY EXPRESSED IN HEMATOPOIETIC CELLS AND LEUKEMIAS AND USES THEREOF

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Moscou et al.

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C07K 14/435  (2006.01)

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OTHER PUBLICATIONS


(74) Attorney, Agent, or Firm—McDermott Will & Emery LLP

ABSTRACT

A novel organic cation transporter (OCT) gene, OCT 6, and use thereof is described. The OCT6 gene is preferentially expressed in human hematopoietic tissues, including CD34+ cells and leukemia cells. Its narrow tissue distribution, substrate specificity, and close homology to other cell membrane transporters make OCT6 an attractive target for the treatment of myeloid diseases.

7 Claims, 10 Drawing Sheets
FIG. 2B
FIG. 4

OCT6 RNA level (relative to MOLT4)

- WBC (unsorted)
- T cells
- B cells
- Monocytes
- Granulocytes
- CD34+ MPB1
- CD34+ MPB2
- CD34+ MPB3
- CD34+ BM
- U937
- THP-1
- KG-1
- MV4-11
- MOLT4
FIG. 5

OCT6 RNA levels (relative to MOLT4)
ORGANIC CATION TRANSPORTER PREFERENTIALLY EXPRESSED IN HEMATOPOIETIC CELLS AND LEUKEMIAS AND USES THEREOF

FIELD OF THE INVENTION

The invention relates to a gene encoding an organic cation transporter, OCT6, and its use as a target for the treatment of hematological malignancies, and in particular, leukemia. The invention further relates to screening methods for identifying agonists and antagonists/binding partners of OCT6 transport activity.

BACKGROUND OF THE INVENTION

The lipid bilayer of the cellular membrane insulates the intracellular milieu from exposure to hydrophilic compounds. Unlike lipophilic compounds that can diffuse through cellular membranes, water-soluble compounds usually require specific transport mechanisms to gain access to the intracellular space. The regulation of the traffic of polar compounds in both directions across the cellular membrane is a complex process involving several large families of transport proteins.

Most often in cancer research, drug transport is thought of as a mechanism of cellular drug resistance, as drug efflux pumps such as the products of the MDR1 and MRP genes have been shown to be mechanisms of resistance to lipid-soluble anticancer drugs. However, drug transport is a two-way street, and mechanisms also exist for pumping drugs into cells. For polar, water-soluble anticancer agents, drug uptake, and not drug efflux, is the critical determinant of cellular drug accumulation.

Most cancer chemotherapy employs drugs that are lipid-soluble that can easily penetrate the cell membrane of cancer cells. One advantage of using lipid-soluble drugs is that they easily gain intracellular access to different types of cancer cells, so many cancer cells appear to be initially sensitive to these drugs. The disadvantage is that cancer cells learn to increase the activity of drug efflux pumps in the cell membrane to pump lipid-soluble drugs out of the cell, resulting in drug resistance.

In contrast, potential water-soluble anticancer drugs may not survive the preclinical screening process since there is a great deal of variability in the expression of drug transport genes in different types of cancer cells. Variability in transport gene expression may result in variability in accumulation of polar, water-soluble drugs. One approach to more effectively utilize water-soluble anticancer drugs is to identify which of the dozens of transport genes are actually expressed in tumors.

The importance of carrier-mediated anticancer drug uptake is exemplified in reduced folate carrier (RFC) mediated uptake of methotrexate (MTX). Methotrexate (MTX), a reduced folate analogue, is scavenged and retained in cells by mechanisms designed to secure folates from the environment. The major mechanism of MTX uptake at pharmacologic concentrations is the reduced folate carrier (RFC), an OAT transporter with a Km for MTX between approximately 0.8-26 μM. Decreased RFC activity has been observed in several in vitro models of transport-mediated MTX resistance (Biochem. Pharmacol. 11: 1233-1234, 1960). Once rodent and human genes encoding proteins with RFC activity were isolated, the molecular explanations for decreased RFC activity emerged. RFC1 transfection into the transport-deficient MTX-resistant ZR75 cell line resulted in a 20-fold increase in 6-hour MTX uptake and a concomitant 250-fold increase in sensitivity to MTX relative to control cell clones, showing that the RFC1 gene reconstitutes RFC activity and has a significant impact on MTX cytotoxicity (Moscow, et al., Cancer Res. 55: 3790-3794, 1995).

In different cell lines, MTX transport deficiency has been ascribed either to mutations in the RFC gene or in decreased expression of the RFC gene product. Several studies have demonstrated that RFC1 gene expression is an important determinant of sensitivity to MTX. In vitro studies, we have found that RFC1 RNA levels correlate with MTX sensitivity in a panel of non-selected cell lines, including breast cancer cell lines (Moscow et al., Int J Cancer. 72: 184-190, 1997).

A plethora of genes with the ability to transport MTX out of the cell have been reported, including MRPL1, MRPL2, MRPL3, MRPL4, the organic anion transporters hOAT1 and hOAT3, and the mitoxantrone-resistance protein (BCRP/MXR). However, despite the multitude of MTX export genes, clinical studies have shown a relationship between the expression of RFC1, the mechanism of MTX uptake, and prognosis in Acute Lymphoid Leukemia (ALL) and osteosarcoma. As a result, RFC1 expression and MTX uptake are now implicated as determinants of clinical sensitivity in several types of tumors. Thus, the role of RFC1 in mediating sensitivity of its cytotoxic drug substrates has become a prototype that illustrates the potential role of transporters, like OAT and OCT genes, in determination of anticancer drug selectivity and toxicity.

However, there is a need to identify additional channels, or transporters, that are found in specific cancers, to enable the targeting of different cancers with anticancer agents that are substrates for those transporters.

SUMMARY OF THE INVENTION

The present invention is directed towards a membrane protein that functions to transport hydrophilic substances across cellular membranes. The protein, OCT6, is a new member of the organic cation transporter (OCT) family (SLC22 gene family). Tissue distribution of this protein is distinct from other OCT protein family members; being detected in leukemia, leukemia blast cells and CD34+ cells.

In one aspect, the present invention provides a novel target for hematological malignancies such as leukemia, an OCT6 transporter.

In another aspect of the present invention there is a method for screening potential substrates that selectively bind the OCT6 transporter. The method involves contacting a cell which overexpresses an OCT6 transporter gene with a test compound and determining whether the test compound is a substrate for the OCT6 transporter.

In another aspect, there is a method for screening potential anti-cancer agents in a cell overexpressing an OCT6 transporter gene. The method comprises determining viability of a cell which expresses OCT6 transporter gene incubated in the presence and absence of a test compound and identifying the test compound as a potential anti-cancer agent if there is cellular influx of the test compound and cell death.
In another aspect of the invention, a test kit is provided for screening candidate drugs for hematologic malignancies comprising a mammalian cell line or cells which overexpress OCT6, a control substrate and a detectable substance.

In still another aspect of the invention, there are immunogenic compositions for treating hematological malignancies. In a preferred embodiment, immunogenic compositions for treating leukemia comprise a substrate that binds selectively to a leukemia cell expressing the OCT6 transporter gene. In another preferred embodiment of the invention, the substrate comprises an antibody that selectively binds to the OCT6 transporter protein. Preferably, the OCT6 transporter protein allows cellular uptake of the substrate which then causes cell death. In one embodiment the substrate is cytotoxic and in another preferred embodiment the substrate is coupled with a cytotoxic agent.

In still another aspect, the present invention provides a method for impairing a leukemia cell comprising contacting the cell with a cytotoxic OCT6 transporter protein. In one embodiment the substrate is a cytotoxic and in another embodiment the substrate is coupled to a cytotoxic agent.

In yet another aspect, the present invention provides a method for treating hematologic malignancies comprising administering to a subject in need thereof an immunogenic composition comprising a substrate that binds selectively to a cell expressing the OCT6 transporter gene. In a preferred embodiment the OCT6 transporter protein allows cellular uptake of the substrate which then causes cell death. In another preferred embodiment the substrate is cytotoxic. In another preferred embodiment, the substrate is coupled with a cytotoxic agent.

**BRIEF DESCRIPTION OF THE DRAWINGS**

**FIG. 1.** A shows the predicted hydropathy profile of OCT6.

**FIG. 1.** B is a dendrogram showing phylogenetic relationship between OCT6 (SEQ ID NO:2) and other OCT and OAT proteins, including, OCTN1 (SEQ ID NO:4), OCT3 (SEQ ID NO:5), OCTN2 (SEQ ID NO:6), OCT2 (SEQ ID NO:7), OCT1 (SEQ ID NO:8), OCT5 (SEQ ID NO:9), OCT4 (SEQ ID NO:10), OCT3 (SEQ ID NO:11), and OAT1 (SEQ ID NO:12).

**FIG. 2A-F.** is the CLUSTALW alignment of OCT6 and other OCT and OAT proteins. The bottom row represents areas of consensus.

**FIG. 3.** shows the normal tissue distribution of OCT6 RNA determined by RT-PCR using a cDNA panel. Only 1000x (highest) cDNA concentration is shown. Panel A. 1. salivary gland; 2. thyroid; 3. adrenal; 4. pancreas; 5. ovary; 6. uterus; 7. prostate; 8. skin; 9. peripheral blood leukocytes; 10. bone marrow; 11. fetal brain; 12. fetal liver. Panel B. 1. brain; 2. heart; 3. kidney; 4. spleen; 5. liver; 6. colon; 7. lung; 8. small intestine; 9. muscle; 10. stomach; 11. testis; 12. placenta.

**FIG. 4.** shows quantitative RT-PCR for the transporter gene OCT6 performed with RNA extracted from peripheral blood leukocytes, CD34+ cells and additional hematopoietic cell lines. Fresh discarded buffy coats that were twice separated by FACS using CD14 (monocytes), CD15 (granulocytes), CD3 (T-cells) and CD20 (B-cells). Purities of 99% or better were obtained. For peripheral WBC and sorted subsets, the average ±SD represent pooled results from samples from 2 individuals performed in triplicate or quadruplicate. For CD34-selected mobilized peripheral blood (MPB), the results from each of 3 individuals are shown. For CD34-selected bone marrow (CD34+ BM), the results are from one individual. OCT6 levels were normalized to the expression of actin RNA, as a control for equivalence of mRNA template. The units, in log scale, are arbitrary and based on a standard curve of OCT6 RT-PCR in serially diluted HL60 RNA. Unity is defined as the level of OCT6 RNA found in MOLT4 cells.

**FIG. 5.** shows quantitative RT-PCR for the gene OCT6 using RNA extracted from leukemia blasts obtained from patients at the time of initial diagnosis. OCT6 levels were normalized to the expression of actin RNA, as a control for equivalence of mRNA template. The OCT6 RNA levels in placenta, liver, kidney and MOLT-4 cell line were determined concurrently and shown for comparison. The units, in log scale, are arbitrary and based on a standard curve of OCT6 RT-PCR in serially diluted HL60 RNA. Unity is defined as the level of OCT6 RNA found in MOLT4 cells.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention is based on the discovery and isolation of a new member of the SLC22 gene family (the OCT family of proteins) that is unusual for its distinct pattern of tissue distribution. Rather than the typical high levels of expression in liver, kidney or placenta, high levels of RNA for this transporter were found in some leukemia cell lines, in CD34+ cells, and in circulating leukemia blasts cells.

All patents, patent applications and literature cited in this description are incorporated herein by reference in their entirety. In the case of inconsistencies, the present disclosure, including definitions, will prevail.

**OCT Family**

Two families of proteins involved in maintaining homoeostasis of charged organic compounds are the organic anion transporters (OATs) which carry the SLC21 designation and the organic cation transporters (OCTs), which carry the SLC22 designation (see Table 1). OATs and OCTs each have characteristic patterns of tissue expression, with predominant expression in a tissue involved in the transport of xenobiotics, i.e., liver, kidney or placenta.

**TABLE 1**

<table>
<thead>
<tr>
<th>Gene Family</th>
<th>Gene Name</th>
<th>Locus Link</th>
<th>Alternative Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC21</td>
<td>SLC21A1</td>
<td>6577</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SLC21A2</td>
<td>6578</td>
<td>GT</td>
</tr>
<tr>
<td></td>
<td>SLC21A3</td>
<td>6579</td>
<td>OATP, OATP1, OATP10, OATP-A</td>
</tr>
<tr>
<td></td>
<td>SLC21A4</td>
<td>28237</td>
<td>OAT-K1, OAT-K2</td>
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<tr>
<td></td>
<td>SLC21A5</td>
<td>28236</td>
<td>OATP2, OATP-2</td>
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<tr>
<td></td>
<td>SLC21A6</td>
<td>10599</td>
<td>LST-1, OATP-C</td>
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<tr>
<td></td>
<td>SLC21A7</td>
<td>28235</td>
<td>OATP3, OATP-3</td>
</tr>
<tr>
<td></td>
<td>SLC21A8</td>
<td>28234</td>
<td>LST2, OATP8, SLC21A8, OATP-8</td>
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<tr>
<td></td>
<td>SLC21A9</td>
<td>11309</td>
<td>OATP-B</td>
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<tr>
<td></td>
<td>SLC21A10</td>
<td>28233</td>
<td>OATP1, OATP5, OATP-5</td>
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<tr>
<td></td>
<td>SLC21A11</td>
<td>28232</td>
<td>OATP-D</td>
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<td>SLC22A5</td>
<td>6584</td>
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<td></td>
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<td>9356</td>
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<td>NLT, OAT2, OAT-2</td>
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<td></td>
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<td>9376</td>
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<td></td>
<td>SLC22A9</td>
<td>6548</td>
<td>OAT4, OAT-4</td>
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</tbody>
</table>

The OAT and OCT carriers result in increased cellular accumulation of their respective substrates, despite the fact
that they are carriers that mediate facilitative diffusion. For carriers, the degree of intracellular accumulation may not exceed the extracellular concentration. However, the presence of the carrier allows uptake in comparison to no uptake in the absence of the carrier, and drugs that bind an intracellular target or which are chemically modified in the cells, e.g., by phosphorylation or polyglutamylation, may be eliminated from the substrate pool and not available for transport back across the cellular membrane.

The first five members of the SLC22 family of transporters, OCT1, OCT2, OCT3, OCTN1, and OCTN2, have been characterized as organic cation transporters. The uptake of many cations, such as tetraethylammonium (TEA), N-1-methyl-Nicotinamide (NMN), choline, procainamide, amantadine and morfine are mediated by these polyspecific transporters. In general, these transporters are potential-dependent, but independent of sodium and proton gradients. These genes are all characterized by the presence of 11 or 12 transmembrane domains, as predicted by hydrophobicity analysis, and all have a large hydrophilic loop between transmembrane domain (TMD) 1 and TMD2.

OCT substrates are shown below in Table 2. Tetraethyl ammonium (TEA) is the classic substrate for OCT transporters. In addition, OCT1, OCT2 and OCT3 transport 1-methyl-4-phenylpyridinium (MPP). Compared to OCT2, OCT1 has a higher affinity for some cations (for example mapiperperidinol and procainamide), a similar affinity for others (for example, decynium 22 and quinidine), and a lower affinity for corticosterone (See Keespall et al., Ann. Rev. Physiol. 60: 243-266, 1998). OCT3 is an electrotransporter for TEA and guanidine. Other physiologic substrates for OCT transporters include dopamine, histamine, epinephrine and norepinephrine, acetylcholine and 5-hydroxytryptamine (Burchardt, et al., Am J Physiol Renal Physiol 278: F853-66, 2000), suggesting an important role for these transporters in the central nervous system, in addition to their role in hepatic and renal clearance. Interestingly, despite its cationic nature, recent studies have identified cimetidine as a selective inhibitor, but not a substrate for several organic cation transporters, including rOCT1, rOCT2, rOCT3, hOCTN1, and hOCTN2.

OCT1 and OCT2 are predominantly expressed in the kidney and liver. These transporters are located on the basolateral surface of renal tubules and, therefore, play a role in the removal of organic cations from the blood. OCT3 is most abundantly expressed in placenta. In addition, other tissue-specific roles have been implicated for these transporters. As noted above, OCTs may play a role in transport of endogenous neurodepletes substances, and OCT3 has been implicated in the disposition of cationic neurotoxins and neurotransmitters in the brain (Wu, et al., J Biol Chem. 273: 32776-86, 1998). Dhillon et al. (Clin Pharmacol Ther. 65: 205, 1999) used RT-PCR followed by functional transport studies (TEA) to identify OCT1 expression in a human mammary epithelial cell line (MCF12A). Further, the OCT1 gene has been shown to be up regulated in lactating mammary epithelial cells.

The OCTN1 gene, cloned from a cDNA, shows sequence similarity to organic cation transporter genes, which is highly expressed in kidney as well as trachea, bone marrow and fetal liver. Recombinant OCTN1 expressed in mammalian cells exhibited saturable uptake of TEA that was pH sensitive. Several others suggest that OCTN1 is a renal proton/organic cation antiporter functioning at the epithelial apical membrane. The uptake of pyrillamine, quinidine, verapamil and L-carnitine were increased by expression of OCTN1 in Xenopus oocytes.

Another OCT protein family member, OCTN2, cloned from a human placent al trophoblast cell line, is expressed widely in human tissues including kidney, placenta and heart. OCTN2 is more closely related to OCTN1 than to OCT1, OCT2 and OCT3 (Biochem Biophys Res Commun. 246: 589-95, 1998). Transection of OCTN2 has demonstrated its role in the transport of TEA and carnitine. OCTN2-mediated transport of TEA is sodium independent, whereas transport of carnitine is sodium-dependent. The role of sodium in OCTN2-mediated carnitine transport not only involves the electrogenic gradient, but the presence of sodium also alters the affinity of OCTN2 for carnitine. Germline mutations of OCTN2 result in primary carnitine deficiency, a syndrome of progressive cardiomyopathy and skeletal myopathy. The symptoms associated with this syndrome are thought to result not from generalized carnitine deficiency from decreased renal carnitine reabsorption, but also from inability of cardiac and skeletal myocytes, which ordinarily express OCTN2, to accumulate carnitine. This syndrome demonstrates that tissue-specific OCT-mediated transport is essential for accumulation of required cations in specific tissues.

The present invention identifies a new transport protein in the OCT family, OCT6, preferentially expressed in leukemia cell lines, leukemia blast cells and CD34+ cells. The cell surface localization and the transporter function of the OCT6 gene product suggest its usefulness as a target in the diagnosis and treatment of hematologic malignancies.

As used herein, the term "antibody" refers to an immunoglobulin molecule with a specific amino acid sequence evoked in by an antigen, and characterized by reacting specifically with the antigen in some demonstrable way.

As used herein, the term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compositions of the present invention are administered.

As used herein, "compound" refers to any agent, chemical, substance, or substrate, whether organic or inorganic, or any protein including antibodies, peptides, polypeptides, polypeptides, and the like.

As used herein, the term "cytotoxin" or cytotoxic agent includes any specific substance, which may or may not be antibody, that inhibits or prevents the functions of cells, causes destruction of cells, or both.

### Table 2

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Gene Name</th>
<th>Cell Type</th>
<th>Substrate</th>
<th>KT (uM)</th>
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<td>SLC22A1</td>
<td>HeLa</td>
<td>TEA</td>
<td>229</td>
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<td>SLC22A1</td>
<td>Xenopus</td>
<td>MPP</td>
<td>14.6</td>
</tr>
<tr>
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<td>SLC22A2</td>
<td>Xenopus</td>
<td>Norpinephrine</td>
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<td>JAR</td>
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</table>
As used herein, the term “derivative” refers to something produced by modification of something pre-existing; for example, a substance or chemical compound that may be produced from another substance or compound of similar structure in one or more steps.

As used herein, the term “fragment” refers to a part of a larger entity, said larger entity comprising by non-limiting example, an antibody, compound or substance.

As used herein, the term “leukemia blast” or “leukemic blast” refers to lymphoblasts, the abnormal immature white blood cells associated with leukemia.

As used herein, the term “monoclonal antibody” is not limited to antibodies produced through hybridoma technology. The term “monoclonal antibody” refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

As used herein, the term “pharmacologically acceptable carrier” refers to a carrier that may be administered to a subject, together with one or more liver protecting agents and one or more mushroom powder or extract of the present invention, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the compound.

As used herein, the term “substrate” refers to a substance, compound, agent, antibody or derivatives and/or fragment thereof, acted upon by the OCT6 transporter protein (e.g., a substance that is taken across the cellular membrane by action of the OCT6 transporter protein).

OCT6 (SEQ ID NO:1) was first identified as a potential OCT gene by assembling and sequencing ESTs as described in Example 1 (amino acid sequence of OCT6 is SEQ ID NO:2). The gene sequence proved to be identical to the recently submitted cDNA OR1B1 (GenBank AF268892) submitted by M. Okabe and T. Imai, incorporated herein in its entirety. It is also contained within the submitted BAC clone CTA-331P3 (SEQ ID NO: 3) (GenBank AC002464) located at chromosome 6q21, incorporated herein in its entirety. The gene has a predicted protein structure typical of transport proteins with two groups of six transmembrane domains separated by a hydrophilic region (FIG. 1A). CLUSTALW alignment produced a dendrogram showing the phylogenetic relationship between OCT6 and other OCT genes (FIG. 1B). This dendrogram suggests that the distinction between OCT genes is shown in FIG. 2 and demonstrates multiple regions of conservation among all of these genes.

Next, according to the methods described in Example 3, quantitative RT PCR analysis of the expression of OCT6 was performed, along with the expression of other OCT genes, in 50 cell lines. The results are shown in Table 3. The two highest expressing cell lines for OCT6 in this panel were two leukemia cell lines, HL60, a human promyelocytic leukemia cell line, and MOLT4, a human acute lymphoblastic leukemia (T-cell) cell line. There was only a low level of expression detected in most of the other cell lines.

### Table 3

<table>
<thead>
<tr>
<th>No.</th>
<th>Cell Line</th>
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<th>OCT1</th>
<th>OCT2</th>
<th>OCT3</th>
<th>OCTN 2</th>
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<td>1</td>
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TABLE 3-continued

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<td>4.2</td>
<td>8.7</td>
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</table>

OCT6 is unique among the known members of OCT and OAT genes because of its pattern of tissue distribution. The pattern of expression of the OCT6 gene in the 50 cell lines suggested that its expression might be restricted to hematopoietic tissues. The restricted pattern of expression observed for OCT6 also suggests that therapies using OCT6-specific substrates are unlikely to have widespread toxicity to normal tissues. Therefore, we examined OCT6 expression in a cDNA panel representing a wide cross-section of normal tissues according to the methods of Example 4 (FIG. 3). This study revealed that OCT6 RNA levels are highest in testis and fetal liver, with lower but detectable levels in peripheral blood leukocytes and bone marrow. Since fetal hematopoiesis occurs in the liver, it is possible that the fetal liver sample may have included both hepatocytes and hematopoietic cells. OCT6 RNA levels were also barely detectable in pancreatic and adrenal tissue. Unlike other OCT genes, expression was not detectable in liver, kidney or placenta.

To determine whether OCT6 RNA expression in hematopoietic cells was lineage-specific, leukocytes were sorted from discarded buffy coat specimens by flow cytometry, and purified subpopulations were examined for OCT6 RNA expression according to the methods described in Example 5. OCT6 expression was also examined in a population of CD34+ cells. As can be seen in FIG. 4, the expression of OCT6 was highly enriched in CD34+ cells in comparison to the other cell populations. Also, significant levels of OCT6 expression (relative to MOLT4) were found in other hematopoietic cell lines: U937, a human histiocytic lymphoma cell line; THP-1, a human acute monocytic leukemia cell line; KG-1, a human erythroleukemia cell line; and MV-4-11, a human biphenotypic (B-cell and myelomonocytic) leukemia cell line.

The high levels of OCT6 RNA in some leukemia cell lines and CD34+ cells also raised the question as to whether this gene was highly expressed in actual leukemias. To address this issue, the RNA levels of OCT6 in 25 samples of peripheral leukemic cells were measured according to the methods set out in Example 6. The FAB classification of these samples are shown in Table 4. These results are shown in FIG. 8, and demonstrate that the majority of specimens contained RNA levels for OCT6 that exceeded the level found in MOLT4 cell line, the second highest expressing cell line among those examined, and exceed by orders of magnitude the levels found in placenta, kidney and liver.

TABLE 4

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Description</th>
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<tr>
<td>1</td>
<td>CML, blast crisis</td>
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<tr>
<td>2</td>
<td>CML, blast crisis</td>
</tr>
<tr>
<td>3</td>
<td>CML, stable phase</td>
</tr>
<tr>
<td>4</td>
<td>CML, probably stable phase</td>
</tr>
<tr>
<td>5</td>
<td>CML, accelerated phase</td>
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Due to the OCT6 protein's location on the cellular membrane and its function as an intracellular transporter, the OCT6 transporter protein has been identified as a therapeutic target. Basic principles of cellular pharmacology suggest that increase in intracellular accumulation will lead to increased intracellular effect. For anticancer drugs, this principle has been studied extensively in the context of lipophilic drugs, which require no specific mechanism for cellular uptake, and export pumps such as the product of the multidrug resistance gene, MDR1, whose overexpression of MDR1 leads to increased cellular resistance by decreasing intracellular concentrations of drug (Moscow, J. A., Schneider, E. S., Ivy, S. P., and Cowan, K. H. Multidrug resistance. In: H. M. Pinedo, D. L. Longo, and B. A. Chabner (eds.), Cancer chemotherapy and biological response modifiers. Annual 17, New York: Elsevier, 1997). The same principle applies to charged, hydrophilic drugs of the present invention, except that the determinants of sensitivity depend on uptake as opposed to efflux. As such, cells expressing an OCT6 transporter are likely to be highly sensitive to cytotoxic OCT6 substrates.
Drug Screening

Accordingly, the present invention provides methods for screening potential substrates of, and potential therapeutic agents against hematological malignancies like leukemia that overexpress, the OCT6 transporter. In particular, potential therapeutic agents are screened for the ability to be a substrate recognized by an OCT6 transporter protein. Preferably, potential substrates are screened for the ability to confer cytotoxic effects on a cell overexpressing OCT6 transporter protein. More preferably, agents are screened for the ability to preferentially cause cellular uptake into, and cell death of, cells overexpressing the OCT6 transporter. Most preferably, the agents are screened for the ability to cause cell death of cancer cells such as leukemia overexpressing the OCT6 transporter as compared to normal cells.

A method for screening potential substrates of the OCT6 transporter protein comprises providing a cell or cell line which expresses OCT6 and a test compound, incubating the test compound and cell line and analyzing the cell or cell line to determine if there was a cellular influx of the test compound. Analysis of the cell line to determine whether cellular uptake of the test compound occurred can be accomplished by any means known in the art. For example, a test compound can be tagged with a detectable label prior to contact with a cell and then observed under microscopy or by other means for its location. Non-limiting examples of labels include green fluorescent protein, alkaline phosphatase, horseradish peroxidase, rease, f3-galactosidase, CAT, luciferase, an immunogenic tag peptide sequence, an extrinsically activatable enzyme, an extrinsically activatable toxin, an extrinsically activatable fluor, an extrinsically activatable quenching agent, a radioactive element or an antibody.

A method for screening candidate anti-cancer agents comprises determining the viability of a mammalian cell which expresses OCT6 incubated in the presence and absence of a test compound and identifying the test compound as a potential anti-leukemia agent if there is a cellular uptake of the test compound and cell death. Analysis of cell viability can be accomplished by any means known in the art. It is well known in the art that viability of a cell can be determined by contacting the cell with a dye and viewing it under a microscope. Viable cells can be observed to have an intact membrane and do not stain, whereas dying or dead cells having “leaky” membranes do stain. Incorporation of the dye by the cell indicates the death of the cell. The most common dye used in the art for determining viability is trypan blue. Viability of cells can also be determined by detecting DNA synthesis. Cells can be cultured in cell medium with labeled nucleotides (e.g., [3H] thymidine). The uptake or incorporation of the labeled nucleotides indicates DNA synthesis and cell viability. In addition, colonies formed by cells cultured in medium indicate cell growth and is another means to test viability of the cells.

Identification and/or observation of cells undergoing apoptosis can be another method of determining cell viability. Apoptosis is a specific mode of cell death recognized by a characteristic pattern of morphological, biochemical, and molecular changes. Cells going through apoptosis appear shrunken, and rounded; they also can be observed to become detached from culture dish. Thermophological changes involve a characteristic pattern of condensation of chromatin and cytoplasm which can be readily identified by microscopy. When stained with a DNA binding dye, such as H33258, apoptotic cells display classic condensed and punctate nuclei instead of homogeneous and round nuclei.

The hallmark of apoptosis is the endonucleolysis, a molecular change in which nuclear DNA is initially degraded at the linker sections of nucleosomes to give rise to fragments equivalent to single and multiple nucleosomes. When these DNA fragments are subjected to gel electrophoresis, they reveal a series of DNA bands which are positioned approximately equally distant from each other on the gel. The size difference between the two bands next to each other is about the length of one nucleosome (i.e., 20 base pairs). This characteristic display of the DNA bands is called a DNA ladder and it indicates apoptosis of the cell. Apoptotic cells can be identified by flow cytometric methods based on measurement of cellular DNA content, increased sensitivity of DNA to denaturation, or altered light scattering properties. These methods are well known in the art and are within the contemplation of the invention.

Abnormal DNA breaks are also characteristic of apoptosis and can be detected by any means known in the art. In one embodiment, DNA breaks are labeled with biotinylated dUTP (b-dUTP). Cells are fixed and incubated in the presence of biotinylated dUTP with either exogenous terminal transferase (terminal DNA transferase assay, TdT assay) or DNA polymerase (nick translation assay; NT assay). The biotinylated dUTP is incorporated into the chromosome at the places where abnormal DNA breaks are repaired, and are detected with fluorescein conjugated to avidin under fluorescence microscopy.

Kits

The present invention provides kits that can be used in the above described methods. In one embodiment, a kit comprises a substantially isolated polypeptide comprising an OCT6 epitope which is specifically immunoreactive with only test compound(s) that are substrates of the OCT6 transporter protein. Binding of a test compound to the OCT6 epitope is indicative that the test compound is an OCT6 substrate. In another embodiment, a kit comprises a cell line that overexpresses an OCT6 transporter protein. Binding and/or cellular uptake of a test compound via the OCT6 protein is indicative that the test compound is a OCT6 substrate. Preferably, the kits of the present invention further comprise a control compound or antibody which does not react with the OCT6 transporter protein. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of a test compound to an OCT6 epitope and/or cellular uptake of a test compound. For example, the test compound may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate.

The detectable substrate may be coupled or conjugated either directly to the test compound (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Pat. No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Further non-limiting examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, nonradioactive paramagnetic metal ions, immunogenic tag peptide sequences, extrinsically activatable toxins, extrinsically activatable quenching agents, or antibodies.

Non-limiting examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/bi-
examples of suitable fluorescent materials include umbellifereone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrins; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include $^{125}$I, $^{131}$I, $^{111}$In or $^{99}$Tc.

Immunogenic Compositions

The present invention also provides immunogenic compositions for the treatment of hematological malignancies. Non-limiting exemplary hematological malignancies include, but are not limited to, Hodgkin’s disease, leukemia such as, acute lymphoid (lymphocytic or lymphoblastic) leukemia (ALL), acute myeloid (myelogenous or myeloblastic) leukemia (AML), acute lymphoid leukemia, biphenotypic (ALL, biphenotypic), acute undifferentiated leukemia (AUL), chronic myeloid (myelogenous or granulocytic) leukemia (CML), erythroleukemia, granulocytic leukemia, lymphoma, monocytes, myeloma, myelomonocytic leukemia, myelodysplastic syndromes, non-Hodgkin lymphoma, progranulocytic leukemia.

According to the invention immunogenic compositions for the treatment of hematological malignancies comprise a substrate recognized by an OCT6 transporter protein. Preferably, the substrate is a compound that binds selectively or specifically to a OCT6 transporter protein. In a preferred embodiment, the compound binds selectively to the OCT6 transporter protein encoded by a nucleotide sequence of SEQ ID NO:1. The compound may be a cytoxin or coupled or conjugated with a cytotoxic agent. Preferably the cytoxin or cytotoxic agent is a chemotherapeutic agent.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier.

Cell surface proteins like the OCT6 transporter can be utilized in antibody-based targeting strategies. In still another aspect of the invention, antibodies can be developed by known methods in the art for the external epitope of OCT6 transporter protein. In a preferred embodiment, antibodies are substrates of the OCT6 protein. The antibodies may be polyclonal antibodies or monoclonal antibodies.

Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecinith, pluronics polyols, polyoxyyn, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guérin) and Corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: a Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entirety).

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate, such as, for example, a linker known in the art, using techniques known in the art. (See, for example, U.S. Pat. No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention.) Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbellifereone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrins; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include $^{125}$I, $^{131}$I, $^{111}$In or $^{99}$Tc.

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, $^{211}$Bi. Non-limiting examples include paclitaxol, cytchalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dillihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and purnomycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., melphalan, thiopea chlorambucil, melphalan, carmustine (BCNU) and lomustine (CCNU), cyclophosphamide, busulan, dibromomannitol, streptozocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DOP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincriistine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response such as inducing cell death for the treatment and prevention of hematological malignancies like leukemia. The therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity for inducing cell death. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomones exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g.,
15 TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi et al., Int. Immunol., 6:1567-1574 (1994)), VEGF (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin, or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Therapeutic Treatment

The present invention is further directed to methods for preventing and treating hematological malignancies such as leukemia. According to the invention, hematological malignancies comprise without limitation, Hodgkin's disease, leukemia such as, acute lymphoid (lymphocytic or lymphoblastic) leukemia (ALL), acute myeloid (myelogenous or myeloblastic) leukemia (AML), acute lymphoid leukemia, biphenotypic (ALL, biphenotypic), acute undifferentiated leukemia (AUL), chronic myeloid (myelogenous or granulocytic) leukemia (CML), erythroleukemia, granulocytic leukemia, lymphocytic leukemia, myeloma, myelomonocytic leukemia, myelodysplastic syndromes, non-Hodgkin lymphoma, progranulocytic leukemia.

Methods of treatment of the present invention comprise administering to a subject in need thereof an immunogenic composition of the present invention. The compositions may be administered with a pharmaceutically acceptable carrier. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannoit, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in “Remington’s Pharmaceutical Sciences” by E.W. Martin. Such compositions will contain in a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is to be administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferrous hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, propranol, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of hematological malignancies can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient’s circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

Various other delivery systems are known and can be used to administer a composition of the invention, e.g., encapsulation in liposomes, microspheres, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (See, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), formation of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intracranial, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucous membranes (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Omnunay reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (See Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Beresteanu and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Beresteanu, ibid., pp. 317-327; see generally ibid.)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biologic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

**EXAMPLES**

The following examples are presented for the illustrative purposes and it is to be understood that the present invention is not limited to those precise embodiments, and that various changes and modifications can be effected therein by one skilled in the art without departing from the scope and spirit of the invention as defined by the appended claims.

**Example 1**

**OCT6 Nucleotide Sequence Identification and Analysis**

OCT6 was first identified as a potential OCT gene by assembling and sequencing ESTs. BLAST searches of human ESTs in GenBank database identified A040384 (654 bp), AA033971 (714 bp) and H70190 (474 bp) sequences from three fetal liver IMAGE clones, 1656502, 429904 and 212935 respectively. IMAGE clone 1656502 (3', insert 1337 bp) ended the predicted stop codon, whereas IMAGE clone 429904 (3', insert 966 bp) and IMAGE clone 212935 (3', insert 966 bp) aligned with the 5'-coding region. All clones were obtained from the IMAGE Consortium through the American Type Culture Collection (Manassas, Va.). Each clone was sequenced in both directions. The sequences were determined using ABI Prism™ 377 DNA sequencer (Perkin-Elmer). Our assemblage proved to be identical to the recently submitted cDNA OKB1 (AF268892) submitted by M. Okabe and T. Abe. We have dubbed this gene OCT6 as OCTN1 and OCTN2 may be considered as OCT4 and OCT5 respectively.

The OCT6 gene (SEQ ID NO:1) is also contained within BAC clone CTA-331P3 (SEQ ID NO:3) (GenBank AC002464) located at chromosome 6q21. It is divided into 6 exons that span 42 kb on the human genome, from nucleotide 79,570 to nucleotide 120490 on CTA-331P3.

**Example 2**

**Molecular Cloning of OCT6**

BLAST searches of human ESTs in GenBank database identified A040384 (654 bp), AA033971 (714 bp) and H70190 (474 bp) sequences from three fetal liver IMAGE clones, 1656502, 429904 and 212935 respectively. IMAGE clone 1656502 (3', insert 1337 bp) ended the predicted stop codon, whereas IMAGE clone 429904 (3', insert 966 bp) and IMAGE clone 212935 (3', insert 966 bp) aligned with the 5'-coding region. All clones were obtained from the IMAGE Consortium through the American Type Culture Collection (Manassas, Va.). Each clone was sequenced in both directions. The sequences were determined using ABI Prism™ 377 DNA sequencer (Perkin-Elmer).

**Example 3**

**Quantitative RT-PCR of OCT6 RNA Levels in Cancer Cell Lines**

Total RNA isolated from 50 cell lines used in the NCI drug screen program was provided by the Developmental Therapeutics Program, NCI. Quantitative RT-PCR for detecting OCT-X transporter gene expression was performed by using a Roche LightCycler, which uses real-time fluorescence detection for quantitative measurement of PCR products. A gene-specific primer pair was designed with Oligo 4.0 software and purchased from Integrated DNA Technologies, Inc. (Coralville, Iowa) (F: 5'-GGCACATTATTCACAAAGCCAAG-3'; (SEQ ID NO: 13) and (F: 5'-TGTGACCTCAAGCAGCATTTGGAT-3') (SEQ ID NO:14). The specificity of the PCR reaction was confirmed by directly determining the DNA sequence of the PCR product. First, cDNA was synthesized from total RNA using SuperScript First-Strand Synthesis System (GIBCO/BRL) in a 20 μl volume following the instructions supplied by the manufacturer. The cDNA treated with RNase H for 20 minutes at 37°C and stored at -20°C. Then, 2 μl of cDNA reaction was amplified in a standard PCR reaction condition, using 0.3 μM primer concentration, with the addition of SYBR Green I Dye. After 30 seconds denaturation at 95°C, the amplification reaction proceeded through 45-50 cycles of 95°C (denaturation for 5 seconds, 62-65°C annealing
for 10 seconds and a 72°C extension for 40 seconds, with slopes of 20°C/s, 20°C/s and 2°C/s, respectively.

Fluorescence was acquired during each cycle after heating to a temperature just below the product melting temperature. Quantification was performed using the LightCycler analysis software. The log-linear portion of the standard amplification curve was identified, and the 'crossing point', a threshold of relative fluorescence, was determined as the best fit through the log-linear region above the background fluorescence (noise) band. The quantification of PCR product was then derived from plotting fluorescence data in the log-linear region of each sample to determine a calculated number of cycles needed to reach the fluorescence crossing point. The calculated number of cycles required to reach the crossing point is proportional to the amount of target RNA in the sample. The relative amount of product was described in arbitrary units by interpolation of the data using a standard curve of a series of dilutions of a standard calibrator RNA. The quantitative measurement of each gene in each cell line was normalized to the relative amount of actin RNA in each cell line, as a control for equivalent cDNA loading in each sample. The results represent the average of 3 independent determinations performed in duplicate.

A melting curve analysis was performed with positive control RNA prior analysis of the cell lines to enhance sensitivity and the specificity of the data. Amplified products usually melt quickly at a temperature characteristic for the products. The fluorescence signal was acquired at a temperature just below the Tm of the specific PCR product and above the Tm of the primer dimers. All specific PCR products displayed a single sharp melting curve with a narrow peak. In addition, PCR products were confirmed for specificity and correct size by visualization of the LightCycler products on a 1% agarose gel.

Example 4

Tissue Distribution

First strand cDNAs derived from 24 adult and fetal tissues (RAPID-SCAN gene expression panel, OriGene Technologies, Rockville, Md.). The PCR primers used in this study were the same as used in the quantitative RT-PCR studies. The PCR reaction samples were denatured at 94°C for 30 seconds, annealed and extended at 64°C for 30 sec for 35 cycles. The PCR products were then visualized on 1% agarose gels.

Example 5

Cell Sorting

All human specimens were obtained in accordance with institutional IRB guidelines. Leukocytes from fresh discarded buffy coats were isolated after RBC lysis with ammonium chloride and labeled with lineage specific antibodies (CD14, monocytes; CD15, granulocytes; CD3, T-cells; and CD20, B-cells), and isolated using a FACSVantage flow cytometer. Each population was sorted twice to ensure purities of at least 99%. CD34 cells were obtained from discarded aliquots of G-CSF-mobilized peripheral blood stem cell collections from cancer patients. For each sample, the PCR results represent the pooled average of cells from 2 individuals performed in triplicate or quadruplicate.

Example 6

OCT6 RNA Levels in Leukemic Blasts

Total RNA was extracted from leukemia specimens using Qiagen RNeasy midi kit. 150 ng of total RNA were used as a template for the first strand cDNA synthesis with the Oligo (dT) primer using the super script system (GIBCO BRL) according to the manufacturer’s protocol. Quantitative real-time RT-PCR was performed using an iCycler thermal cycler with methods similar to those described above for the Roche LightCycler. The results represent the average of 3 independent determinations performed in duplicate.

Although illustrative embodiments of the present invention have been described in detail, it is to be understood that the present invention is not limited to those precise embodiments, and that various changes and modifications can be effected therein by one skilled in the art without departing from the scope and spirit of the invention as defined by the appended claims.

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<211> LENGTH: 551
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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 35  40  45
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 50  55  60
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 65  70  75  80
Cys Ser Arg Tyr Arg Leu Ala Thr Ile Ala Ann Phe Ser Ala Leu Gly
 85  90  95
Leu Glu Pro Gly Arg Asp Val Asp Leu Gly Glu Leu Glu Glu Ser
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<210> SEQ ID NO 6
<211> LENGTH: 557
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

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Gly Phe Thr Gly Leu Ser Ser Val Phe Leu Ile Ala Thr Pro Glu His  
35  40  45

Arg Cys Arg Val Pro Asp Ala Ala Asn Leu Ser Ser Ala Thr Pro Asn  
50  55  60

His Thr Val Pro Leu Arg Leu Arg Asp Gly Arg Gly Glu Val Pro His Ser  
65  70  75  80

Cys Arg Arg Tyr Arg Leu Ala Thr Ala Asn Phe Ser Ala Leu Gly  
85  90  95

Leu Glu Pro Gly Arg Asp Val Asp Leu Gly Gin Leu Glu Gin Glu Ser  
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Cys Leu Asp Gly Trp Glu Phe Ser Gin Asp Val Tyr Leu Ser Thr Ile  
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Val Thr Glu Trp Asn Leu Val Cys Glu Asp Asp Trp Lys Ala Pro Leu  
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<210> SEQ ID NO 7
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 7

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Ile Gly Arg Arg Tyr Pro Trp Ala Ala Ser Asn Met Val Ala Gly Ala
405 410 415
Ala Cys Leu Ala Ser Val Phe Ile Pro Gly Asp Leu Gln Trp Leu Lys
420 425 430
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435  440  445
Ile Val Cys Leu Val Asn Ala Glu Tyr Thr Pro Phe Ile Arg Asn
450  455  460
Leu Gly Val His Ile Cys Ser Ser Met Cys Asp Ile Gly Gly Ile Ile
465  470  475  480
Thr Pro Phe Leu Val Tyr Arg Leu Thr Asn Ile Trp Leu Glu Leu Pro
485  490  495
Leu Met Val Phe Gly Val Leu Gly Leu Val Ala Gly Gly Leu Val Leu
500  505  510
Leu Leu Pro Glu Thr Lys Gly Ala Leu Pro Glu Thr Ile Glu Glu
515  520  525
Ala Glu Asn Met Gin Arg Pro Arg Asn Lys Lys Glu Lys Met Ile Tyr
530  535  540
Leu Gln Val Glu Lys Leu Asp Ile Pro Leu Asn
545  550  555

<210> SEQ ID NO 8
<211> LENGTH: 554
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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20  25  30
Ala Pro Ile Cys Val Gly Ile Val Phe Leu Gly Phe Thr Pro Asp His
35  40  45
His Cys Gln Ser Pro Gly Val Ala Glu Leu Ser Gin Arg Cys Gly Trp
50  55  60
Ser Pro Ala Glu Glu Leu Asn Tyr Thr Val Pro Gly Leu Gly Pro Ala
65  70  75  80
Gly Gln Ala Pro Leu Gly Gln Cys Arg Arg Tyr Gly Val Asp Trp Asn
85  90  95
Gln Ser Ala Leu Ser Cys Val Asp Pro Leu Ala Ser Leu Ala Thr Asn
100 105 110
Arg Ser His Leu Pro Leu Gly Pro Cys Gin Asp Gly Trp Val Tyr Asp
115 120 125
Thr Pro Gly Ser Ser Ile Val Thr Glu Phe Asn Leu Val Cys Ala Asp
130 135 140
Ser Trp Lys Leu Asp Leu Phe Gin Ser Cys Leu Asn Ala Gly Phe Leu
145 150 155 160
Phe Gly Ser Leu Gly Val Gly Tyr Phe Ala Asp Arg Phe Gly Arg Lys
165 170 175
Leu Cys Leu Leu Gly Thr Val Leu Val Asn Ala Val Ser Gly Val Leu
180 185 190
Met Ala Phe Ser Pro Asn Tyr Met Ser Met Leu Phe Arg Leu Leu
195 200 205
Gln Gly Leu Val Ser Lys Gly Asn Trp Met Ala Gly Tyr Thr Leu Ile
210 215 220
Thr Gly Phe Val Gly Ser Gly Ser Arg Arg Thr Val Ala Ile Met Tyr
225 230 235 240
Gln Met Ala Phe Thr Val Gly Leu Val Ala Leu Thr Gly Leu Ala Tyr
245 250 255
Ala Leu Pro His Trp Arg Trp Leu Gln Leu Ala Val Ser Leu Pro Thr
260 265 270
Phe Leu Phe Leu Leu Tyr Tyr Trp Cys Val Pro Glu Ser Pro Arg Trp
275 280 285
Leu Leu Ser Gln Lys Arg Asn Thr Glu Ala Ile Lys Ile Met Asp His
290 295 300
Ile Ala Gln Lys Asn Gly Lys Leu Pro Pro Ala Asp Leu Lys Met Leu
305 310 315 320
Ser Leu Glu Glu Asp Val Thr Glu Leu Ser Pro Ser Phe Ala Asp
325 330 335
Leu Phe Arg Thr Pro Arg Leu Arg Lys Arg Thr Phe Ile Leu Met Tyr
340 345 350
Leu Trp Phe Thr Asp Ser Val Leu Tyr Glu Leu Ile Leu His Met
355 360 365
Gly Ala Thr Ser Gly Asn Leu Tyr Leu Asp Phe Leu Tyr Ser Ala Leu
370 375 380
Val Glu Ile Pro Gly Ala Phe Ile Leu Ile Thr Ile Asp Arg Val
385 390 395 400
Gly Arg Ile Tyr Pro Met Ala Met Ser Asn Leu Leu Ala Gly Ala Ala
405 410 415
Cys Leu Val Met Ile Phe Ile Ser Pro Asp Leu His Trp Leu Asn Ile
420 425 430
Ile Ile Met Cys Val Gly Arg Met Gly Ile Thr Ile Ala Ile Gln Met
435 440 445
Ile Cys Leu Val Asn Ala Glu Leu Tyr Pro Thr Phe Val Arg Asn Leu
450 455 460
Gly Val Met Val Cys Ser Ser Leu Cys Asp Ile Gly Gly Ile Ile Thr
465 470 475 480
Pro Phe Ile Val Phe Arg Leu Arg Glu Val Trp Glu Ala Leu Pro Leu
485 490 495
Ile Leu Phe Ala Val Leu Gly Leu Ala Ala Gly Val Thr Leu Leu
500 505 510
Leu Pro Glu Thr Lys Gly Val Ala Leu Pro Glu Thr Met Lys Asp Ala
515 520 525
Glu Asn Leu Gly Arg Lys Ala Lys Pro Lys Glu Asn Thr Ile Tyr Leu
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545 550

<210> SEQ ID NO 9
<211> LENGTH: 539
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 9

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Gln Met Leu His Leu Val Phe Ile Leu Pro Ser Leu Met Leu Leu Ile
20 25 30
Pro His Ile Leu Leu Glu Asn Phe Ala Ala Ala Pro Gly His Arg
35 40 45
Cys Trp Val His Met Leu Asp Asn Thr Gly Ser Gly Asn Gly Thr
50 55 60
Gly Ile Leu Ser Glu Asp Ala Leu Leu Arg Ile Ser Ile Pro Leu Asp
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355 360 365
Asp Phe Leu Gly Arg Ala Thr Thr Ala Leu Leu Leu Ser Phe Leu Gly
370 375 380
Arg Arg Thr Ile Gln Ala Gly Ser Gln Ala Met Ala Gly Leu Ala Ile
385 390 395 400
Leu Ala Asn Met Leu Val Pro Gln Asp Leu Gln Thr Leu Arg Val Val
405 410 415
Phe Ala Val Leu Gly Lys Gly Cys Phe Gly Ile Ser Leu Thr Cys Leu
420 425 430
Thr Ile Tyr Lys Ala Glu Leu Phe Pro Thr Pro Val Arg Met Thr Ala
435 440 445
Arg Gly Ile Leu His Thr Val Gly Arg Leu Gly Ala Met Met Gly Pro
450 455 460
Leu Ile Leu Met Ser Arg Gln Ala Leu Pro Leu Leu Pro Pro Leu Leu
465 470 475 480
Tyr Gly Val Ile Ser Ile Ala Ser Ser Leu Val Leu Phe Phe Leu
485 490 495 500
Pro Glu Thr Gln Gly Leu Pro Leu Pro Asp Thr Ile Gln Asp Leu Glu
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Ser Gln Lys Ser Thr Ala Ala Gln Gly Asn Arg Glu Glu Ala Val Thr
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<210> SEQ ID NO 11
<211> LENGTH: 542
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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20  25  30
Asn His Asn Leu Leu Gln Ile Phe Thr Ala Ala Thr Pro Val His His
35  40  45
Cys Arg Pro Pro His Asn Ala Ser Thr Gly Pro Trp Val Leu Pro Met
50  55  60
Gly Pro Asn Gly Lys Pro Glu Arg Cys Leu Arg Phe Val His Pro Pro
65  70  75  80
Asn Ala Ser Leu Pro Asn Asp Thr Glu Arg Ala Met Glu Pro Cys Leu
85  90  95 100
Asp Gly Trp Val Tyr Asn Ser Thr Lys Asp Ser Ile Val Thr Glu Trp
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What is claimed is:

1. A method of screening candidate substrates of the
   organic cation transporter 6 (OCT6) comprising:
   a. providing a test agent;
   b. providing mammalian cells or a mammalian cell line
      which express OCT6;
   c. incubating the test agent with the cells or cell line; and
   d. determining whether the test agent is a substrate for
      OCT6,
   wherein the mammalian cells or mammalian cell line
   provided in step b are leukemia cells or a leukemia cell line,
   respectively.

2. The method of claim 1 wherein the test agent is coupled
to a detectable substance.

3. The method of claim 2 wherein the detectable substance
   is selected from the group consisting of extrinsically activatable
   enzymes, prosthetic groups, fluorescent materials, luminescent
   materials, bioluminescent materials, radioactive materials,
   positron emitting metals using various positron emission
tomographies, nonradioactive paramagnetic metal ions,
immunogenic tag peptide sequences, extrinsically activatable
   toxins, extrinsically activatable quenching agents, and antibodies.

4. The method of claim 1 wherein the step of determining
   whether the test agent is a substrate for OCT6 comprises
   analyzing whether the test agent is located intracellularly.

5. The method of claim 1, wherein step (d) comprises
   determining the viability of the cells or cell line.

6. The method of claim 5, wherein the viability of the cells
   or cell line is determined by applying a dye to the cells or cell
   line, wherein incorporation of the dye by the cells is indicative
   of death of the cells or cell line.

7. The method of claim 6, wherein the dye is trypan blue.

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