The serotonin transporter (SLC6A4) is present in B-cell clones of diverse malignant origin: probing a potential anti-tumor target for psychotropics

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SPECIFIC AIMS

We recently described the presence of a functioning serotonin transporter (SERT; SLC6A4) in the constituent cells of Burkitt's lymphoma. Here we address the frequency of SERT expression among a broad spectrum of B cell malignancy and probe the potential of both prescribed and recreational psychotropics that selectively target the transporter as anti-tumor therapeutics.

PRINICPAL FINDINGS

1. SERT is expressed in derived B cell lines of diverse malignant origin

Seventeen derived B cell lines of diverse tumor origin were analyzed for SERT expression. In terms of B cell differentiation stage, a spectrum from pre-B to plasma cell was covered with the inclusion of lines derived from B cell precursor acute lymphoblastic leukemia (BCP-ALL) through to multiple myeloma (MM). Burkitt's lymphoma (BL), pro-lymphocytic leukemia (PLL), mantle cell lymphoma (MCL), diffuse large B cell lymphoma (DLBCL), and primary mediastinal B cell lymphoma (PMBCL) were also represented. Western blot analysis revealed readily detectable SERT appearing as two dominant bands of apparent molecular masses of ~60 and 70 kDa in all 17 lines (**Fig. 1*A***). These bands were present in HEK 293 cells transfected with full-length neuronal SERT (HEK-SERT), these cells additionally carrying a diffuse 90–95 kDa species representing extensively glycosylated protein. Specific peptide blocking confirmed the specificity of all 3 immunoreactive species (Fig. 1*B*). Normal tonsilar B cells contained no detectable immunoreactive SERT in the case of germinal center cells or undetectable to low levels in the case of the resting, extrafollicular population. Mitogenic stimulation by phorbol ester (PMA) and a calcium ionophore (CaI) led to the induction of readily detectable SERT in the resting B cells (Fig. 1*C*). A novel FACS-based assay confirmed the presence of immunoreactive SERT in all the B cell lines with regression analysis indicating a linear correlation between the amount of protein expressed and basal proliferation rate (r=0.643; P<0.001).

2. Psychotropic drugs that target SERT promote apoptosis in model Burkitt's lymphoma cell lines

Serotonin (5-hydroxytryptamine; 5-HT) and antidepressants of the selective serotonin reuptake inhibitor (SSRI) class have been shown to promote apoptosis in SERT-carrying BL cells. We now add to the serotonergic compounds affecting BL population dynamics the tricyclic antidepressant (TCA) clomipramine and the amphetamine derivatives 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") and fenfluramine (FEN, an appetite suppressant). MDMA and FEN behaved similarly to 5-HT in their ability to arrest growth of the model L3055 BL line with an IC_{50} for each compound of 100–200 μM at 24 h, which did not alter substantially by day 3 or 6 of culture. In contrast, the TCA clomipramine followed the behavior of the SSRI fluoxetine where an IC_{50} of ~10 μM at 24 h reduced to ~ 1 μM by day 6. The nonselective

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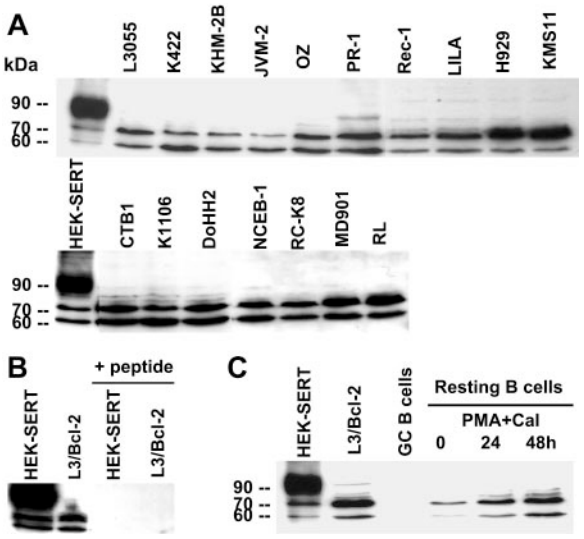

Figure 1. 10 μg of protein from HEK293 cells transfected with full-length neuronal SERT (HEK-SERT) was resolved on the gels compared with 50 μg from B cells and B cell lines before probing with anti-SERT. *A)* Lysates prepared from B cell lines. *B)* Samples from HEK-SERT and L3055/Bcl-2 transfectants (L3/Bcl-2) blotted with anti-SERT in absence or presence of blocking peptide. *C)* Lysates form germinal center (GC) B cells or resting B cells stimulated with PMA (5 ng/mL) and calcium ionophore (CaI; 1 μg/mL) ionomycin for times shown.

biogenic amine transporter antagonist cocaine was also found to be antiproliferative for BL cells but with a significantly higher IC_{50} and requiring concentrations of >500 μM to achieve appreciable effect. For clomipramine and the amphetamine derivative FEN, their capacity to growth-arrest the L3055 BL line was shown to be accompanied by concentration-dependent increases in the proportion of nonviable cells, in cells displaying caspase activity, and in cells recognized as early apoptotic by possessing increased caspase activity while excluding propidium iodide.

3. A majority of derived B cell lines demonstrate an antiproliferative response to psychotropics

5-HT, amphetamine derivatives MDMA and FEN, SSRI fluoxetine, TCA clomipramine, and cocaine were assessed for potential antiproliferative effect against the different B cell lines. Concentrations of each compound were selected according to maximum/near maximum effects against the model L3055 BL cells; representative outcomes at 24 h of exposure are illustrated in **Fig. 2**. Of the 17 lines, 12 revealed an antiproliferative response to one or more of the compounds at the ≥50% level. In general, sensitivity to 5-HT was reflected in the response to FEN and, to a slightly lesser extent, MDMA. Eight of the lines showed a ≥50% antiproliferative response to 20 μM clomipramine, with half of these responding similarly to equimolar fluoxetine. No line was substantially affected by 1 mM cocaine. When measuring viability at 24 h, normal resting (tonsilar) B cells were not adversely affected by any of the serotonergic compounds used.

4. Bcl-2 protects from proapoptotic but not antiproliferative actions of psychotropics whereas P-glycoprotein expression appears not to influence outcome

Bcl-2 and P-glycoprotein (P-gp) levels can each influence the course of a malignant B cell's response to potential therapeutic drugs while amphetamine derivatives and antidepressants have been reported to interact directly with the latter. We assessed the expression of both parameters in the B cell lines by quantitative FACS analysis. Only 2 of 17 malignancies expressed significant P-gp, among which one was sensitive (Rec-1) to the anti-tumor actions of psychotropics and the other refractory (RC-K8). A range of Bcl-2 levels was found with all 5 lines carrying the t(14;18)(q32; q21) translocation involving *bcl*-2 and the Ig heavy chain gene locus being relatively high expressors, as expected. To assess formally the impact of high and/or dysregulated Bcl-2 expression on the anti-lymphoma response to psychotropics, *bcl*-2 was introduced as a constitutive transgene into model L3055 BL cells, which resulted in protection from the proapoptotic actions of the serotonergic compounds tested. The antiproliferative response, however, remained intact. Malignancies carrying the t(14;18)(q32;q21) translocation were similarly resistant to apoptosis induction by psychotropics yet could still succumb to proliferation arrest.

CONCLUSIONS AND SIGNIFICANCE

This study establishes SLC6A4/SERT expression as a phenotype common to neoplastic B cell clones of widely distinct tumor origin. SERT was present at appreciably higher levels among the constituent cells of malignant lines compared with normal B cell populations but could be induced in the latter on (potent) mitogenic stimulation. Assessing first against model BL cells, the amphetamine derivatives MDMA and FEN

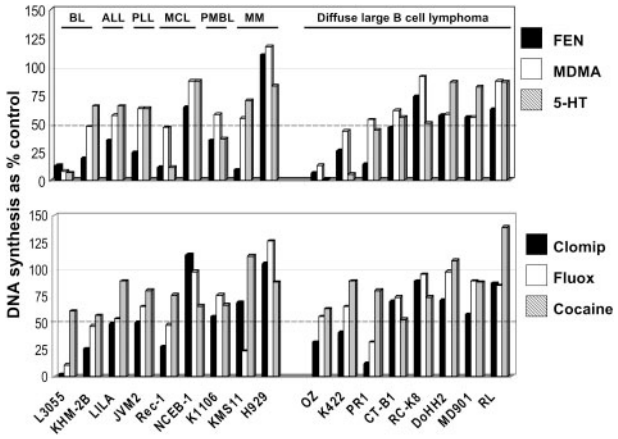

Figure 2. Results shown as DNA synthesis (mean of 3 experiments) in response to compound as % of control (untreated) cultures after 24 h. FEN and MDMA at 500 μM; 5-HT, 250 μM; clomipramine (Clomip) and fluoxetine (Fluox) at 20 μM; cocaine, 1 mM.

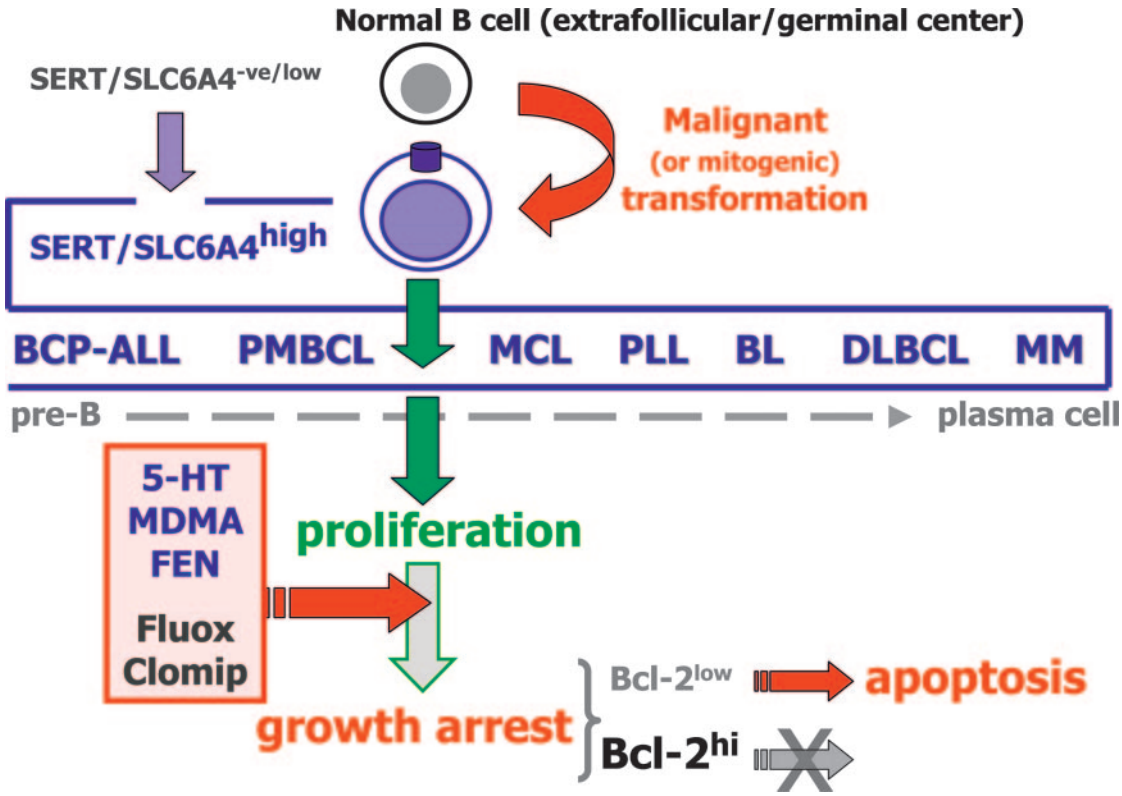

Figure 3. Expression of SERT/SLC6A4 and its potential as a therapeutic target in B cell malignancy. Normal B cells in their basal state are low/negative for SERT expression but strong induction occurs on malignant (or mitogenic) transformation. Neoplastic B cell clones are susceptible to growth arrest from serotonergic compounds: SERT providing the target for 5-HT and amphetamine derivatives while SSRI and TCA antidepressants may act by mechanisms independent of the transporter. Growth arrest is followed by apoptosis, where Bcl-2 levels are low.

were found to be equipotent to the natural SERT substrate 5-HT in driving proliferation arrest and apoptosis. This is encouraging as 5-HT is not itself a practical therapeutic drug. Though the concentrations of MDMA and FEN required to elicit an anti-tumor effect are high, their efficacy nonetheless indicates a potential for amphetamine analogs in this novel context: one perhaps reached by redesigning "designer drugs"? Of more immediate promise as therapeutics for B cell malignancy are the antidepressants. Profound anti-lymphoma effects in vitro, particularly on prolonged exposure, were registered with an SSRI (fluoxetine) and a TCA (clomipramine) at concentrations known to be reached in the serum of patients on standard dosing regimes for anxiety-related disorders. Among the spectrum of malignancies studied, 8 of 17 responded significantly to one or both of the antidepressants. Though high/dysregulated Bcl-2 expression could protect from apoptosis-induction by psychotropics, it failed to confer resistance to proliferation arrest. Strategies designed to knockdown *bcl*-2 in these situations would likely further improve their efficacy.

The universal presence of SLC6A4 among the malignant B cell clones described here and the equipotent anti-tumor actions of the two SERT-acting amphetamine analogs and 5-HT strongly indicate the transporter as a potential therapeutic target in lymphoid neoplasia. Though initially providing the rationale for their inclusion in this study we feel it unlikely that the efficacy of either fluoxetine or clomipramine against malignant B cells is focused solely, or even primarily, on SERT. The antiproliferative actions of the two antidepressants require concentrations that are supra-saturating with regards to the serotonin transporter. We have found that their effect is mimicked fully by maprotiline and GBR12909: selective uptake inhibitors at the transporters for norepinephrine and dopamine, respectively. A common feature of all 4 compounds is that they are strong cationic amphiphiles. This contributes to the potent lipophilicity of the drugs with a correspondingly high partition to tissues through phospholipid binding and lysosomal trapping. Each property would not only account for their capacity to influence cell dynamics but also makes them particularly suited to crossing the blood-brain barrier: These drugs concentrate strongly in the brain compared with the periphery. We would therefore suggest that, as is currently being studied in trial with clomipramine for brain tumors, TCA and SSRI antidepressants would offer particularly exciting prospects for the treatment of CNS lymphoma. While the evolution and origin of primary CNS lymphoma remains a mystery, like all non-Hodgkin's lymphoma, it continues to increase in frequency and is predominantly a B cell disease. FJ