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ABCB5 gene amplification in human leukemia cells

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Efficacy of cancer chemotherapy may be impaired by tumor resistance to multiple, structurally unrelated therapeutic drugs with different mechanisms of action, a phenomenon termed multidrug resistance (MDR). One mechanism of MDR is decreased intracellular drug accumulation resulting from expression of the ATP-dependent drug efflux transporter P-glycoprotein (P-gp, ABCB1) or related ATP-binding cassette (ABC) transporters, including the recently identified novel P-gp family member ABCB5. ABCB5 regulates membrane potential and cell fusion of skin progenitor cells and mediates resistance to doxorubicin, 5-FU and camptothecin in human malignant melanoma in cells of cancer stem cell (CSC) phenotype and function. Relatively little is known regarding the expression and function of the ABCB5 gene in additional physiological and malignant tissues. In this issue of Leukemia Research, Lehne et al. report ABCB5 gene amplification and enhanced ABCB5 gene expression in multidrug resistant human K562 leukemia cells. Intriguingly, upregulated expression of the CSC marker ABCB5 is associated with en bloc activation of hematopoietic and leukemic stem cell genes, indicating that ABCB5 might represent a chemoresistance mechanism in leukemic stem cells.

In order to identify genes associated with multidrug resistance acquired by human K562 chronic myelogenous leukemia (CML) cells through vincristine (VCR) selection, Lehne et al. performed comparative genomic hybridization (CGH) and gene expression profiling of drug resistant $K562_{VCR}$ cells compared to sensitive wildtype (wt) $K562_{wt}$ cultures. CGH revealed amplification of the human ABC transporter genes *ABCB1*, *ABCB4* and *ABCB5* in $K562_{VCR}$ compared to $K562_{wt}$ cells, and these gene amplifications were associated with overexpression of the respective mRNAs in resistant $K562_{VCR}$ cells. In addition, expression microarrays demonstrated upregulation of stem cell genes (e.g. *KIT*, *HOXB4*) and anti-apoptotic genes (e.g. *IGF1R*, *CCNG1*), as well as downregulation of pro-apoptotic genes (e.g. *CASP4,6* and *7*) in

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drug-resistant versus sensitive K562 cells, indicating that acquisition of multidrug resistance correlated with the emergence of a stem cell phenotype in K562 CML cells.

Increases in genomic DNA copy number through gene amplification with resultant alterations in gene expression have been associated with tumor progression and drug resistance in hematologic malignancies and solid tumors [1]. Furthermore, the observed correlation of ABC transporter expression with the emergence of a stem cell phenotype in CML parallels the preferential overexpression of ABC transporters on physiological haematopoietic stem cells and cancer stem cells (CSC) in acute myeloid leukemia (AML), where physiological or malignant CD34+CD38- stem cells were shown to express higher levels of ABC transporter genes, including *ABCB1*, *ABCB4* and *ABCB5*, compared to more differentiated, physiological or malignant CD34+CD38+ cells, respectively [2].

The expression of ABC transporters in stem cells may predominantly relate to their established functions in cellular xenobiotic and drug efflux transport, which might serve to confer protection from environmental toxins and provide mechanisms for chemotherapeutic resistance to these more quiescent and long-lived cell subsets. For example, ABCB1 confers MDR through mediating the cellular efflux of multiple neutral and hydrophobic cationic compounds and chemotherapeutic agents, including the chemotherapeutic drug vincristine [3] used for selection of resistant $K562_{VCR}$ cells. ABCB4 also exhibits drug transport capacity, but to a more limited spectrum of agents than ABCB1 [3]. ABCB5 is a chemoresistance mediator first identified and characterized in human skin [4] and human malignant melanoma [4–6]. Among skin-derived cultured human epidermal melanocytes (HEM), ABCB5 was found to mark cells of $CD133^+$ phenotype, a multipotent stem cell population in human skin [7]. As a determinant of membrane potential, ABCB5 was shown to regulate cell fusion of this stem cell subset with more differentiated cell types, thereby contributing to HEM culture growth and differentiation [4]. In human malignant melanoma, ABCB5 was also found preferentially expressed on $CD133^+$ cells[5]. Moreover, ABCB5 was shown to mediate doxorubicin resistance in these stem cell phenotype-expressing melanoma subpopulations via its function as a drug efflux transporter [5]. Huang et al. identified additional roles of ABCB5 in melanoma chemoresistance to camptothecin and 5-FU [8]. Importantly, ABCB5 was shown to identify CSC in human malignant melanoma that correlate with clinical disease progression and could be targeted through ABCB5 to inhibit tumor growth in preclinical melanoma models, providing proof-of-principle for the potential therapeutic utility of the cancer stem cell concept [6]. In addition, the findings of this study provided evidence for a novel, potentially critical link between CSC, chemoresistance and cancer progression, raising the possibility that $ABCB5^+$ CSC may be responsible both for the progression and chemotherapeutic refractoriness of advanced malignant disease, and that CSC-targeted approaches might therefore represent novel and translationally relevant therapeutic strategies to disseminated melanoma growth [6]. In other human cancers, the role of ABCB5 is currently unknown. However, the intriguing observation by Lehne et al. that development of drug resistance in a CML cell line results in ABCB5 gene amplification and mRNA overexpression and in the induction of stem cell genes raise the possibility that ABCB5 may serve similar functions in this malignancy, with potentially important therapeutic implications should similar mechanisms be operative in drug-resistant clinical leukemias.

It should be noted that the ABCB5 gene locus is complex and that more work is therefore needed to define the ABCB5 transcript(s) overexpressed in $K562_{VCR}$ CML cells. According to AceView [9] alone, 80 GenBank accessions from 69 cDNA clones indicate that ABCB5 gene transcription can produce at least 11 different ABCB5 mRNAs (a-k) including 9 alternatively spliced variants and 2 unspliced forms. Of those, the tissue expression patterns of ABCB5.a (also referred to as ABCB5β [10]), an 812 a.a. (amino acid) transporter [4,5], and of ABCB5.f (also referred to as ABCB5α [10]), a molecule of unknown function [4], have been reported, with mRNA

expression detected at high levels predominantly in human skin and malignant melanoma [4, 5,8,10] (Table 1). In addition, ABCB5.a is expressed at the mRNA level in additional physiological tissues including the central nervous system, in testis, colon, stomach, mammary gland and retina, and in additional human malignancies including cancers of the breast, lung, colon, and stomach [5]. In contrast, ABCB5.e, also cloned and sequenced by our laboratory from murine and human skin (GenBank Accession Numbers AY766238 and AY785909, respectively) is ubiquitously expressed in physiological and malignant tissues, including in K562 leukemia cells [11,12] (Table 1). Moreover, an additional mRNA transcript, encoding a tandem repeat transporter consisting of 1255 a.a. (mouse, AY766239) or 1257 a.a. (human, AB353947) and comprising two transmembrane and nucleotide binding domains, named ABCB5.ts (testis-specific), has been identified, amplified and sequenced by our laboratory, with expression of its full open reading frame selectively restricted to testis tissue [13] (Table 1).

Given these diverse possibilities of ABCB5 gene transcription, it remains presently unknown if ABCB5 is expressed in vincristine-selected human CML cells as a functional drug efflux transporter and mediator of multidrug resistance, or if alternative ABCB5 variants are induced as a result of vincristine selection, with potential transport-independent functions associated with a stem cell phenotype of resistant leukemia cells. However, the results by Lehne et al. provide a clear rationale and an experimental model system to further address these possibilities and to initiate mechanistic studies regarding the potential functions of ABCB5 in human CML.

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Table 1

ABCB5 expression variants in human and murine tissues

	GENBANK ACCESSION NUMBER		PREDICTED PROTEIN (AMINO ACIDS)		PRINCIPAL TISSUE EXPRESSION		
ABCB5 VARIANT	**Human**	**Mouse**	**Human**	**Mouse**	**Human**	**Mouse**	**REFERENCES**
ABCB5.a	AY234788		812 a.a.		Skin, melanoma		4’5’10
ABCB5.e	AY785909	AY766238	134 a.a.	134 a.a.	Ubiquitous	Ubiquitous	11’12
ABCB5.f	BC044248		131 a.a.		Skin, melanoma		4’8’10
ABCB5.ts	AB353947	AY766239	1257 a.a.	1255 a.a.	Testis	Testis	13