ABC Transporters: Maintenance of the Cancer Stem Cell Phenotype

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Abstract: The poor therapeutic response to anti-cancer treatment and inferior prognosis of carcinoma primarily result from cancer stem cells (CSCs), which initiate and maintain tumors. Recent studies have demonstrated that the molecular phenotype of CSCs mainly consists of multidrug resistance (MDR), self-renewal, multi-lineage differentiation potential (pluripotency) and tumorigenicity. Intriguingly, ATP-binding cassette (ABC) membrane transporters are highly expressed in CSCs compared to non-CSCs, and recent evidence has highlighted a link between ABC transporters and the CSC phenotype. Understanding the relationship between CSCs and ABC transporters is important as this could lead to the development of more efficacious treatment regimens. Thus, in this article, we will mainly review the relationships between ABC transporters and the phenotype of CSCs.

Keywords: ABC transporters, cancer stem-like cells (CSCs), multidrug resistance (MDR), self-renewal, pluripotency, side population (SP), tumorigenicity.

INTRODUCTION

In recent years, although great progress has been made in the treatment of against cancer, the refractory status of cancer, including chemo-resistance, recurrence and metastasis, remains a major challenge. The understanding of the causes responsible for the failure of anticancer agents, recurrence and metastasis of tumors is of great importance. Emerging studies have shown that cancer stem-like cells (CSCs) play a key role in these processes [1-6]. Malignant stem-like cells have been identified in various solid tumor and leukemia. Similar to normal stem cells, CSCs are able to self-renew, differentiate, and proliferate extensively [7, 8]. The cancer mass that originates from rare stem-like cells can transfer the disease to immunodeficient mice, suggesting that these cancer stem-like cells (CSCs) are responsible for relapse following conventional or targeted cancer therapy and that eradication of CSCs may be necessary to cure the disease permanently. However, current therapeutic strategies may not effectively ablate the CSCs, leaving the potential for disease progression or relapse.

The ATP binding cassette (ABC) transporters are ubiquitous membrane proteins, consisting of both transmembrane domains (TMDs) and distinctive nucleotide-binding domains (NBDs), which generate energy from ATP hydrolysis to actively transport a variety of compounds across the membrane [9-11]. Based on sequence homology and domain organization of the TMDs, the ABC family of transporters can be divided into seven distinct subfamilies (ABCA-ABCG), which are further divided into sub-subfamilies (except the ABCE/OABP family) [12, 13] (Table **1**).

One of the important physiological roles of most of these proteins is to pump out diverse endogenous substrates including sugars, amino acids, peptides, proteins, and other hydrophobic compounds using the energy of ATP hydrolysis [10, 11]. In addition, these transporters catalyze the efflux of numerous xenobiotics, including antineoplastic drugs, thereby protecting normal tissues from cytotoxic effects [14, 15].

The overexpression of ABC drug transporters confers cross-resistance to multiple drugs belonging to different chemical classes *via* active efflux and thus, reducing the intracellular levels below that required to produce a therapeutic effect, resulting in MDR [16]. About 13~14 out of 49 members of the ABC protein family may produce MDR in cancer cells. The most important members in mediating MDR are ABCB1/P-gp, ABCG2/BCRP and ABCC1/MRP1 [11, 15, 17].

Currently, in addition to MDR, a large body of evidence indicates that there are some links between ABC transporters and the CSC phenotype [18, 19]. The elucidation of the relationship between ABC transporters and CSCs may provide a basis for improving therapeutic interventions against malignant cancer. Therefore, in this paper, we review the relationships between ABC transporters and the CSC properties including MDR, self-renewal, pluripotency and tumorigenicity.

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Table 1: Classification of the Human ABC Transporter Family and Members that Determine MDR

Subfamily	Alternative name	Number of proteins	Proteins that confer drug resistance	
			Name	Number
ABCA	ABC1	12	ABCA2	1
ABCB	MDR	11	ABCB1(P-gp)	3
			ABCB4(MDR3)	
			ABCB11(BSEP,SPGP)	
ABCC	MRP	13	ABCC1(MRP1)	9
			ABCC2-6(MPR2-6)	
			ABCC10(MPR7)	
			ABCC11(MRP8)	
			ABCC12(MRP9)	
ABCD	ALD	4		
ABCE	OABP	1		
ABCF	GCN20	3		
ABCG	White	5	ABCG2(BCRP)	1
Total		49		14

1. ABC TRANSPORTERS AND MDR OF CSCs

The failure of cancer chemotherapy can occur through acquired-resistance or intrinsic resistance to antineoplastic drugs [20, 21]. The nature of clinical drug resistance is multifactorial, involving alterations in drug targets, inactivation/detoxification of the drug, decreased drug uptake, increased drug efflux, and the dysregulation of apoptotic pathways [22]. The reduction of intracellular drug levels can be mediated by an increased expression of specific ATP binding cassette (ABC) transporters, which are responsible for multidrug resistance (MDR) [23]. The most extensively characterized ABC transporters that mediate MDR are ABCB1 (also known as MDR1 or P-glycoprotein), ABCC1 (also known as MRP1) and ABCG2 (also known as BCRP or MXR) [24].

1.1. ABCB1 and MDR of CSCs

ABCB1 (P-gp), a 170-kDa transmembrane glycoprotein from the superfamily of ATP binding cassette transporters, serves as an ATP-dependent efflux pump for a variety of chemicals, including many antineoplastic agents such as taxanes, anthracyclines and vinca alkaloids [25]. Furthermore, numerous studies indicate that the overexpression of ABCB1 is associated with multidrug resistance (MDR) [15, 26, 27].

Cancer stem-like cells are defined as “a small subset of cancer cells within a cancer that constitute a reservoir of self-sustaining cells with the exclusive ability to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor” [28]. Currently,, there is considerable evidence indicating that ABCB1 transporters are overexpressed in various types of CSCs [29-36]. Interestingly, these cancer stem-like cells are resistant to a variety of conventional therapies [37]. Therefore, ABCB1 may be responsible for the MDR of CSCs, mainly through the efflux of antineoplastic drugs.

There are several mechanisms that may mediate the ABCB1-induced MDR of CSCs: (1) overexpression of ABCB1; (2) functional abnormality in ABCB1 [38, 39]; (3) the presence of subcellular P-gp [40]; (4) inhibition of cisplatin-induced caspase-3 activation [41]; (5) modulation of intracellular calcium homeostasis [42-46];

1.2. ABCC1 and MDR of CSCs

Apart from P-gp, multidrug-associated protein 1 (MRP1 or ABCC1) also plays a role in the development of drug resistance in the majority human cancers, including those of the lung, breast and prostate, as well as childhood neuroblastomas [47]. The ABCC1 transporter was the first member of the MRP family reported to be linked with MDR [48]. It catlyzes the efflux of antineoplastic drugs such as anthracyclines and mitoxantrone, as well as drugs conjugated to glutathione- (GSH), sulfate- or glucuronate [49].

Numerous studies have shown MRP1 is overexpressed in a variety of solid tumors and MRP1 mediates drug resistance in several types of cancers [47]. A study by Marques *et al.* found that Lucena cells resistant to chemotherapy (also expressing $CD34^{+}CD38^{-}$) significantly overexpressed ABCC1 genes [50]. Moreover, Vesuna *et al.* found the first direct evidence demonstrating that increased ABCC1 transporter levels in Twist-overexpressing cells lead to the development of chemo-resistance and development of the stem cell phenotype in these cells [51]. In addition, both *in vitro* and *in vivo* studies have reported that down-regulation of MRP1 in cancer cell lines or tumor xenografts produces significant chemosensitization to clinically relevant MRP1 substrate drugs, thereby verifying its role in MDR [52-54].

1.3. ABCG2 and MDR of CSCs

ABCG2 (BCRP, breast cancer resistance protein, ABCP or MXR) was first identified in a MCF-7/Adr-Vp3000 cancer cell line that neither expressed P-gp nor MRP1 but exhibited a high resistance to doxorubicin, mitoxantrone and daunorubicin [55]. The mediation of chemoresistance by ABCG2 was first reported by Zhou *et al.* who demonstrated that stem cells derived from $ABCG2^{-}$ deficient mice were more sensitive to mitoxantrone, an antioneoplastic drug that is a substrate for ABCG2 [56]. Numerous *in vitro* studies indicate that the overexpression of the ABCG2 transporter is correlated with MDR in various types of cancers [57]**.** Furthermore, a study by Bhatia *et al.* reported that ABCG2 is expressed in the nuclear extracts of select glioblastoma and astrocytoma cell lines as well as in a human ABCG2 tumor biopsy [58].

Overall, ABCG2 and P-gp play a critical role in chemoresistance in CSCs or SP cells, though the detailed relationship between them remains unknown. ABC transporters- mediated MDR involves not only the expression level and functional activity of ABC transporters, but also the subcellular location of ABC transporters. Recent studies suggest that ABC transporter-mediated MDR may also occur by their interaction with certain cell regulatory pathways [59], down-regulation of cell surface saccharide targets [60], interaction with intracellular calcium homeostasis and by regulating endoplasmic reticulum proteins [42, 61].

2. ABC TRANSPORTERS AND SELF-RENEWAL CAPACITY OF CSCs

Self-renewal is the process where a stem cell divides to generate one (asymmetric division) or two (symmetric division) daughter stem cells indistinguishable from those of the mother cells, while preserving their developmental potentials [62]. Unlike normal stem cells or embryonic stem cells, cancer stem cells have an unlimited self-renewal capability [62]. Recently, multiple lines of evidence indicate that self-renewal is regulated by a variety of signal transduction pathways, different transcription factors, and many molecular events. These include the expression of Sox-2 [63, 64], Oct-4 [65-69], Nanog [70], Myc [71], Bmi-1 [72-79] and the stem cell signaling pathways: Notch [80, 81], Wnt [82-86] and Hedgehog [87, 88]. Additionally, recent evidence indicates that transcription factors associated with self-renewal pathways modulate the expression of some ABC transporters, and thus maintain the self-renewal capability of cancer stem cells [89] (Figure **1**). For example, Marques *et al.* suggested that ABCB1 may be responsible for self-renewal capacity differences between the K562 and Lucena cell line, due to the Oct-4-induced changes in P-gp expression in the Lucena cell line [50]. Also, recent *in vitro* experiments in esophageal and prostate cancer cell lines suggest that the Hh pathway increases the expression of ABC transporters P-gp and ABCG2 [90]. In addition, the knockdown of transcription factor Gli decreased the expression of BCRP and P-gp [90, 91]. Moreover, the translocation of Gli increases the expression of ABCB1, ABCC1, and ABCG2 [92]. The aforementioned studies suggest that there is an association between the aberrant self-renewal related pathways and the overexpression of ABC transporter Currently, the specific relationship between ABC transporters and the capacity of self-renewal in CSCs remains unknown. However, there are several postulated mechanisms by which ABC transporters could produce self-renewal in CSCs. For example, it is possible that ABCG2 expression could reduce the accumulation of DNA-damaging metabolites in mouse embryonic stem cells (ESCs), which helps prevent cell differentiation but maintain self-renewal [93]. An increase in the level of ABCG2 was observed in the colony expansion of embryonic stem (ES), followed by a decrease in the level of protoporphyrin IX (PPIX) [75]. These results suggest that ABCG2 plays a role in preventing porphyrin accumulation in ES cells and excessive production of ROS from heme metabolism during colony expansion [94] (Figure **2**). Overall, based on the current experimental evidence, it is possible that self-renewal-related pathways (e.g. Hegdehog) and transcription factors could modulate self-renewal process through regulating ABC transporters.

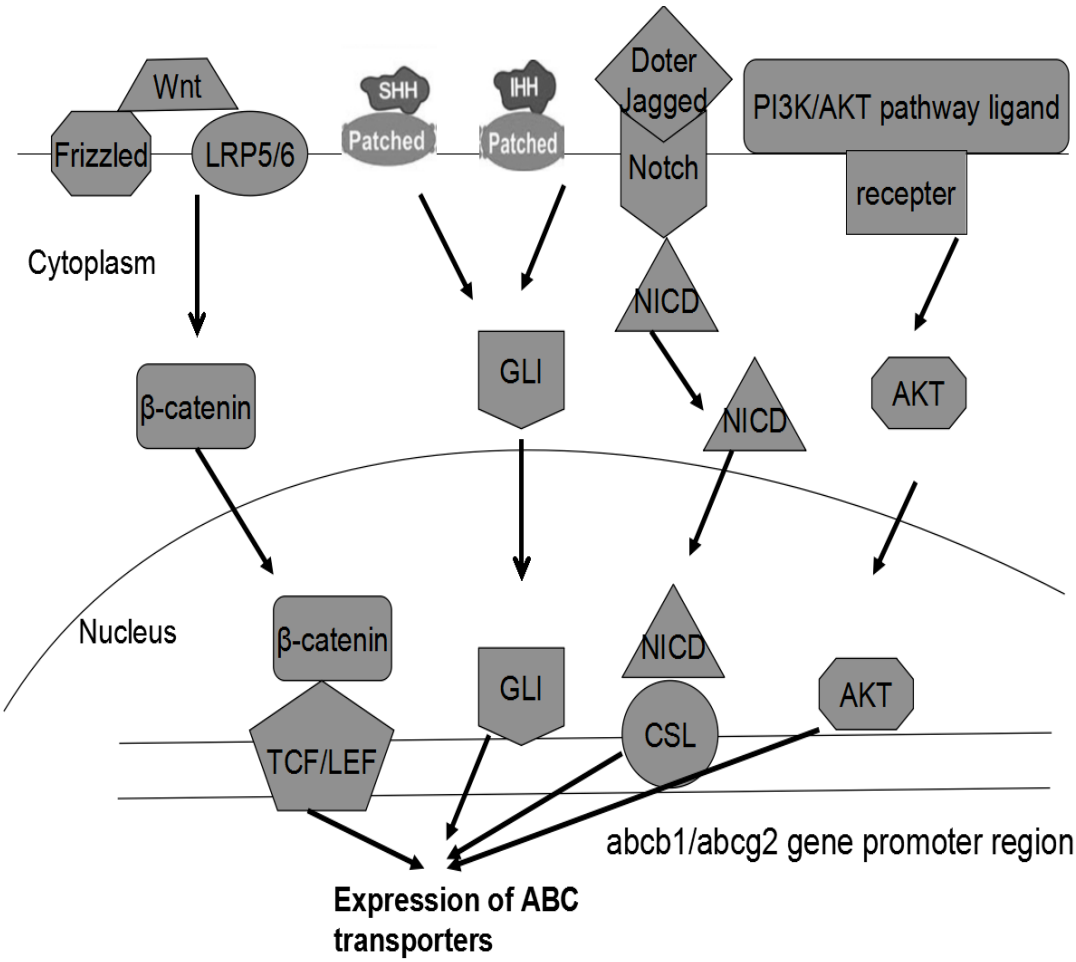

Figure 1: The effects of CSCs associated pathways on the expression of ABC transporters. ABC transporters have been shown to be regulated by the Hedgehog, Notch, and Wnt pathways. The activation of the Wnt pathway releases β-catenin from a repression complex and it translocates to the nucleus, where it interacts with the TCF/LEF consensus binding sites on the basal promoter of ABC transporter genes, which leads to the their expression. Also, when the ligand SHH or IHH act with Patched, the Hedgehog pathway is activated. And its effector GLI translocates to the nucleus and bonds to the promoter of ABC transporters, which result in the expression of ABC transporters. In addition, when Doter and jagged bond to the Notch complex, an active component named NICD (Notch intracellular domain) is generate and it interacts with the CSL of promoter of the ABC transporters, thereby increasing their expression. The activation of the PI3K/AKT pathway also can produce the expression of ABC transporters through the binding of AKT with the ABC transporter gene promoter.

Therefore, the relationship between self-renewal and ABC transporters may provide a novel therapeutic strategy for overcoming the maintenance and survival of cancer stem cells.

3. ABC TRANSPORTERS AND PLURIPOTENCY OF CSCs

Cancer stem-like cells possess the ability to generate all types of cancer cell types (pluripotency), which form the heterogeneous macroscopic tumors [95]. Recent studies have revealed that some stem cell-like markers, such as Nanog, Oct3/4, c-Myc and Sox-2, are associated with pluripotency [68, 69, 96, 97]. Similarly, multiple lines of evidence indicate that ABC transporters, such as ABCB1 and ABCG2, are markers of cancer stem–like cells, and may be responsible for some key traits of cancer stem-like cells, such as the self-renewal and MDR [56, 98]. However, to date, little is known about the detailed interaction of ABC transporters and pluripotency of cancer stem-like cells. Recent studies have indicated that ABC transporters may play a specific role in the maintenance of pluripotency of some types of normal tissue stem/progenitor cells, as indicated by the fluctuation in the expression of ABC transporters during differentiation and regeneration of different kinds of normal tissue stem/progenitor cells [99-101]. For example, a study by Juuti-Uusitalo K *et al.* found that the gene expression of MRP1, -3, -4, -5, and P-gp fluctuated during hESC-RPE (human stem cell-derived retinal pigment epithelial cells) maturation from undifferentiated hESC to fusiform, epithelioid, and

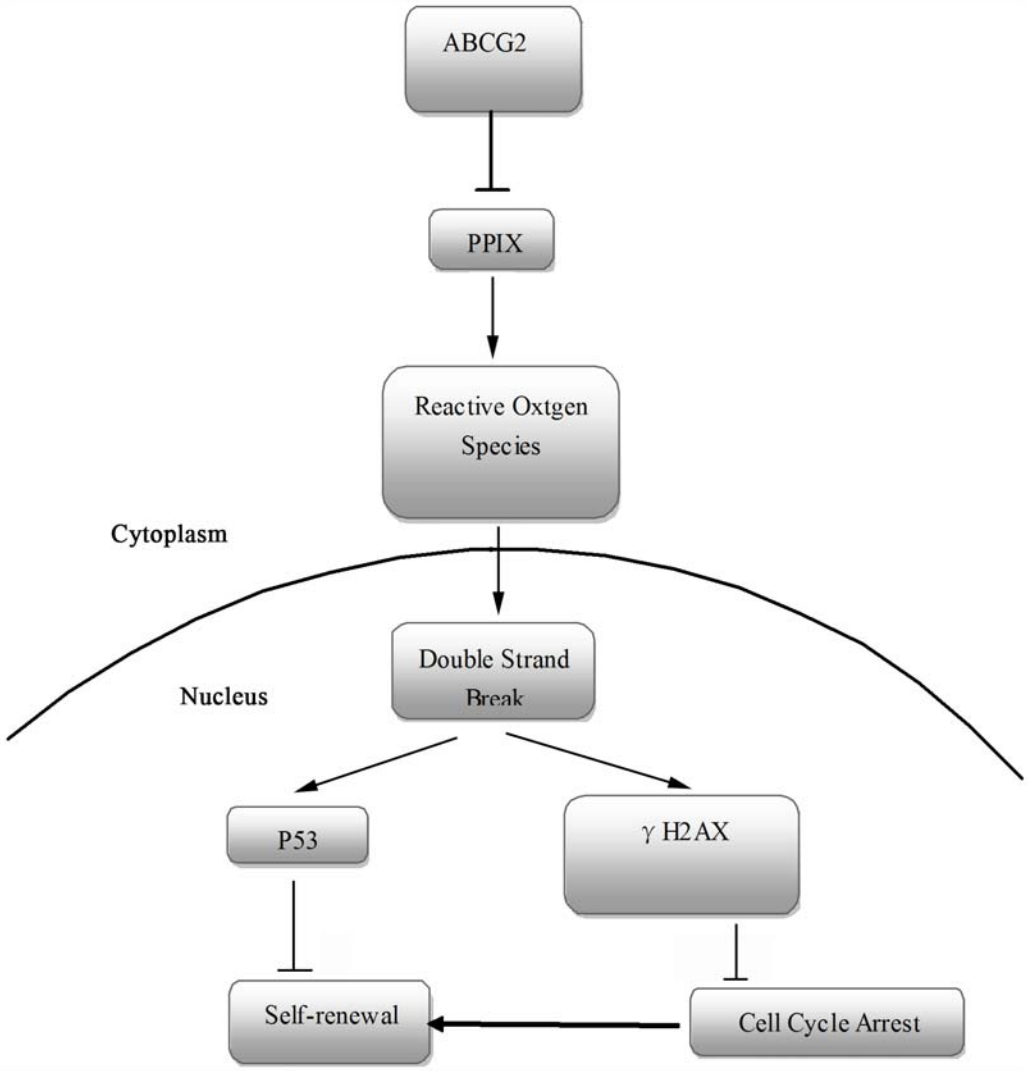

Figure 2: The possible mechanism of ABCG2 mediated self-renewal of CSCs. ABCG2 plays a role in preventing the accumulation of porphyrin and DNA damaging agents which triggers DNA damage signals such as Patm, pp53 and γH2AX.

finally to cobblestone hESC-RPE [99]. Furthermore, Islam *et al.* found that ABCB1 expression decreased during human fetal neural stem/progenitor cells (hNSPCs) differentiation and that ABCB1 may maintain hNSPCs in an undifferentiated state and could be a neural stem/progenitor marker [101]. More importantly, Barbet *et al.* reported that the expression of the 49 human ATP binding cassette (ABC) genes (except ABCB5, ABCC11, ABCC12, ABCG5, ABCG8) in pluripotent embryonic stem cells and in early- and late-stage multipotent mesenchymal stem cells may play a role in maintaining human stem cell pluripotency [100]. A recent study has shown that ABC transporters contribute to both local and global regulation of cAMP, a ubiquitous second messenger that affects multiple cell functions from maturation of the egg to cell division, growth, differentiation, and death [102]. However, the evidence discussed above is correlative and definitive studies are needed to 1) determine whether the expression of ABC transporters is associated with the pluripotency of cancer stem cells and 2) determine the mechanism.

4. ABC TRANSPORTERS AND TUMORIGENICITY OF CSCs

The ABC transporters appear to play an important role in tumorigenicity, as there are studies indicating a positive correlation between the expression of several ABC transporters and tumorigenicity (Table **2**). However, there are also published studies indicating either no correlation or a negative correlation between the expression of ABC transporters and tumorigenicity (Table **2**).

For example, clinical observations and genetic data from mouse models demonstrate that ABCB5 may

Table 2: Effects of ABC Transporters on Tumorigenicity in Tumor Stem-Like Cells

ABC Transporters	Effects on Tumorigenicity	Cancer types	Ref.
ABCB1	Positive	breast cancer, uveal melanoma	[103, 104]
ABCB5	Positive	Melanoma, colorectal cancer, oral squamous cell carcinoma	[19, 105-107]
ABCB6	Positive	hepatocellular carcinoma	[108]
ABCC4	Positive	pancreatic cancer	[109]
ABCG2	Positive	Gliomas, gallbladder cancer, ovarian cancer	[110-112]
	Negative	nasopharyngeal carcinoma	[113]
	NE	cell lines (U373 glioma and MCF7 breast cancer) and a xenograft prostate tumor (LAPC-9)	[114]

Note: ABC, ATP-binding cassette; Positive, promoting tumorigenicity; Negative, inhibiting tumorigenicity; NE, no effect on tumorigenicity.

contribute to the high tumorigenic capacity of several kinds of human cancer stem-like cells [19, 105-107]. In serial human-to-mouse xenotransplantation experiments by Tobias Schatton *et al.* [19], ABCB5+ melanoma cells were shown to have a greater tumorigenic capacity than ABCB5- bulk populations. Martin Grimm *et al.* reported that ABCB5 expression in oral squamous cell carcinoma cells may be associated with tumor formation [106]. Moreover, a $ABCB5^+$ melanoma subpopulation can trigger tumorigenesis through enhanced self-renewal [107]. In addition to ABCB5, there are some studies shows that ABCC4, P-gp and ABCB6 could also promote the process of tumorigenicity of pancreatic cancer cells, breast cancer cells and uveal melanoma cells, respectively [104, 108, 109]. In general, side population cells (mainly mediated by ABCG2) always possess a higher tumorigenic capacity relative to the non–side population cells. However, an *in vivo* study by Lubna Patrawala *et al.* found that although side population cells show an increased ABCG2 mRNA expression relative to the non–side population cells, highly purified $ABCG2^+$ cancer cells have tumorigenicity similar to the $ABCG2^-$ cancer cells [114]. Therefore, the higher tumorigenicity of the side population cells may be resulted from the combined effects of several other subpopulations of cells, in addition to the $ABCG2^+$ cells. For example, cells expressing other ABC family members, such as MDR1 and MRP1 may also contribute to the cancer cell side population phenotype. However, it has been reported that the cloning efficiency of $ABCG2^+$ cells was lower than that of $ABCG2^-$ cells and unsorted cells, and the tumorigenic capacity of $ABCG2^+$ cells was also the lowest. These results may have been due to the presence of other components that enrich side populations and thus there are non-side populations in $ABCG2^+$ cells and there are some side populations in $ABCG2^-$ cells. In addition, $ABCG2^+$ cells are not equal to side population cells and therefore do not exhibit typical properties of tumor stem-like cells.

Together, these studies indicate that the expression profile of ABC transporters may play a fundamental role in the process of tumorigenesis. However, to date, most of the evidence is largely correlative and further studies are needed to uncover the definite relationship between ABC transporters and tumorigenesis.

5. FUTURE DIRECTION

Currently, the clinically used antineoplastic drugs primarily target rapidly proliferating tumor cells, and have virtually no effect on cancer stem cells, thereby leading to tumor recurrence and metastasis. Therefore, the development of treatment regimens that target cancer stem cells could significantly decrease the likelihood of therapeutic failure and relapse. We have reviewed studies reporting that certain ABC transporters may play a role in maintaining the CSC phenotype. In our opinion, the effective treatment of cancer should involve the treatments that not only reduce the number of proliferating cancer cells but also ones that target CSCs. However, additional research will be required to determine the mechanism(s) by which ABC transporters mediate the self-renewal property of CSCs. We believe that as the mechanism is clarified step by step, the treatment of malignant neoplastic disease will be more effective.

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