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Minireview

ABCG2: A potential marker of stem cells and novel target in stem cell and cancer therapy

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ABSTRACT

ABCG2 is a member of the ATP binding cassette (ABC) transporters, which can pump a wide variety of endogenous and exogenous compounds out of cells. Widely expressed in stem cells, ABCG2 is also found to confer the side population phenotype and is recognized as a universal marker of stem cells. Although the precise physiological role of ABCG2 in stem cells is still unclear, existing data strongly suggest that ABCG2 plays an important role in promoting stem cell proliferation and the maintenance of the stem cell phenotype. In addition, ABCG2 is also found to be expressed in a number of cancer cells and appears to be a marker of cancer stem cells. Moreover, ABCG2 expression in tumors may contribute to their formation and progression. Thus, ABCG2 has potential applications in stem cell and tumor therapy.

Contents

Introduction

ATP binding cassette (ABC) transporters form one of the largest transmembrane protein families. These proteins use cellular ATP to drive the transport of various substrates across cell membranes including drugs, metabolites and other compounds. To date, about 50 human ABC transporters have been identified in a variety of mammalian

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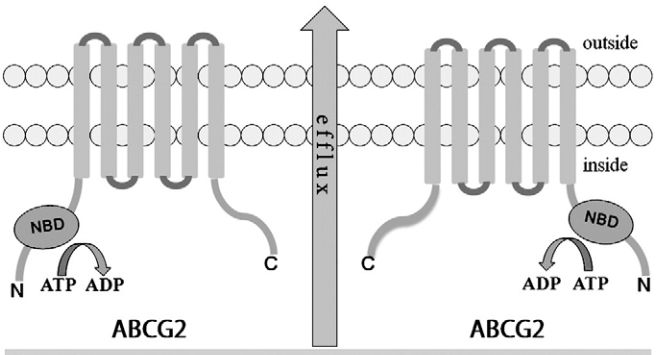
cells (Schinkel and Jonker 2003). Based on the arrangement of component domains, human ABC transporters are divided into seven subfamilies (from A to G). Human ABCG2 is the second member of the G subfamily of ABC transporters. *ABCG2* was first cloned from doxorubicin-resistant human MCF-7 breast cancer cells and named as breast cancer resistance protein (BCRP) (Doyle et al. 1998). Shortly after, other teams reported two nearly identical genes termed *ABCP* (placental ABC protein) (Allikmets et al. 1998) and *MXR* (mitoxantrone resistance protein) (Miyake et al. 1999). Following the cloning of *BCRP*/*ABCP*/*MXR*, the Human Genome Nomenclature Committee suggested that the transporter be renamed ABCG2. ABCG2 is widely distributed in normal tissues and is highly expressed in a subpopulation of stem cells: the side populations (SP), which were first described in bone marrow by their ability to efflux Hoechst 33342, the DNA binding dye (Zhou et al. 2001). Its conserved expression in stem cell populations suggests an important role in stem cell biology. In addition, ABCG2 is one of the most important multidrug-resistance transporters and its substrates include many commonly used drugs in cancer chemotherapy (Robey et al. 2007). There is increasing evidence that ABCG2 correlates with unfavorable prognosis in a variety of tumors (Ross and Nakanishi 2010). ABCG2 may impact cancer treatment outcomes through active efflux of anticancer drugs or other mechanisms (Ross and Nakanishi 2010). Recent studies suggest that ABCG2 may be involved in cancer stem cells (CSCs) (Dean et al. 2005). In this review, we will summarize the current knowledge about ABCG2 with respect to its expression and function in stem cells and tumors. Finally, the potential role of ABCG2 in the characterization of cancer stem cells is discussed.



Chemotherapy agents	imatinib	BODIPY-prazosin
mitoxantrone	erlotinib	rhodamine 123
flavopiridol	**Natural substrates**	Lyso-Tracker Green
9-aminocamptothecin	PhIP	**Others**
topotecan	phosphatidylserine	antivirals
irinotecan	pheophorbide α	antibiotics
SN-38	PPIX	HMGCoA reductase inhibitors
methotrexate	**Fluorescent dyes**	flavonoids
gefitinib	Hoechst 33342	

Fig. 1. Structure of the ABCG2 transporter and a list of its substrates. ABCG2 is a half-transporter, which consists of a single nucleotide binding domain (NBD) and a single six transmembrane domain, functioning as a homodimer. It hydrolyzes cellular ATP to transport diverse substrates ranging from chemotherapeutic agents to fluorescent dyes across a cell membrane.

ABCG2 gene, structure and substrates

The human *ABCG2* gene maps to chromosome 4q22, spans over 66 kb and consists of 16 exons and 15 introns. Its coding protein contains 655 amino acids (72 kDa). ABCG2 is a half-transporter, requiring dimerization to become functionally active. Unlike other ABC half-transporters, which are usually expressed in cellular membranes, ABCG2 localizes predominantly to the plasma membrane (Rocchi et al. 2000).

Since ABCG2 was first described in drug-resistant cell lines, it has been established that ABCG2 has the capacity to transport a broad range of substrates. Typical chemotherapy agents transported by ABCG2 include mitoxantrone, flavopiridol, 9-aminocamptothecin, topotecan, irinotecan and its active metabolite SN-38, methotrexate and the tyrosine kinase inhibitors gefitinib, imatinib, and erlotinib (Polgar et al. 2008). ABCG2 has also been shown to play a role in the transport of natural substrates such as 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP), phosphatidylserine, pheophorbide α, and protoporphyrin IX (PPIX) (van Herwaarden et al. 2003; Woehlecke et al. 2003; Robey et al. 2004; Jonker et al. 2007). In addition, ABCG2 is proved to be responsible for the efflux of fluorescent dyes Hoechst 33342 and BODIPY-prazosin (Robey et al. 2003). Rhodamine 123 and Lyso-Tracker Green are substrates of ABCG2 when amino acid 482 is mutated (Robey et al. 2003). Several other substrate classes including antivirals, antibiotics, HMGCoA reductase inhibitors and flavonoids have been described to be transported by ABCG2 (Polgar et al. 2008). For protein structure and a list of ABCG2 substrates, see Fig. 1.

Regulation of ABCG2 expression

To date, the molecular mechanisms regulating the expression of ABCG2 remain unclear. An estrogen response element was previously identified in the *ABCG2* promoter (Ee et al. 2004b). However, conflicting data are shown to impact the ABCG2 expression for estrogen (Ee et al. 2004a,b; Imai et al. 2005; Wang et al. 2006). Other steroid hormones such as progesterone, human placental lactogen and human prolactin have been shown to have stimulatory effects on ABCG2 expression in human placental choriocarcinoma BeWo cells (Wang et al. 2008a,b). In addition, folate has been found to induce ABCG2 expression, while folate deprivation results in the loss of ABCG2 expression (Ifergan et al. 2004). ABCG2 expression is up-regulated by hypoxia and injury via hypoxia-inducible transcription factor HIF-1 and HIF-2α signaling, respectively (Krishnamurthy et al. 2004; Martin et al. 2008). In drug-resistant MCF-7 cells, alternative use of the 5′ promoter due to differential expression of splice variants at the 5′ untranslated region (UTR) of *ABCG2* mRNA has been observed (Nakanishi et al. 2006). DNA methylation and histone modifications were reported to play important roles in the epigenetic regulation of ABCG2 expression in human renal carcinoma and multidrug-resistant cells, respectively (To et al. 2006, 2008a). Furthermore, To et al. (2008b) identified a putative microRNA binding site in a portion of the 3′UTR and suggested that a putative microRNA binds at this site and can suppress expression of ABCG2. Pan et al. (2009) recently reported that microRNA-328 transfection in MCF-7 cells targets the 3′UTR of *ABCG2* and decreases ABCG2 expression.

Cytokines and growth factors have also been shown to alter ABCG2 expression. Evseenko et al. (2007) showed that treatment of primary term trophoblasts with tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) markedly decreased ABCG2 expression. On the other hand, epidermal growth factor (EGF) and insulin-like growth factor II significantly increased ABCG2 expression. When ABCG2-positive MCF-7 side population cells were treated with transforming growth factor-beta (TGF-β), Yin et al. (2008) detected decreased *ABCG2* gene expression.

Finally, signaling pathways that control ABCG2 expression have been described.

The activation of the Sonic Hedgehog (SHH) and notch signaling pathways which are critical in stem cell and tumor biology, has been shown to promote the expression of ABCG2 (Sims-Mourtada et al. 2007; Bhattacharya et al. 2007). Mogi et al. (2003) demonstrated that the serine/threonine protein kinase Akt positively modulated ABCG2 activity. This Akt-induced ABCG2 activation results from its translocation to the plasma membrane. Takada et al. (2005) have also shown that the phosphorylation state of Akt regulates cell surface expression of ABCG2 in the porcine renal endothelial LLC-PK1 cells. Recently, PTEN/PI3K/Akt pathway has been shown to regulate ABCG2 activity in glioma cancer stem-like cells. Inhibiting the PI3K/Akt pathway strongly decreased the activity of the transporter, while loss of the Akt

inhibitor PTEN increased the SP phenotype (Bleau et al. 2009). Differential ABCG2 regulation was observed downstream of the MEK–ERK pathway. ABCG2 is transcriptionally up-regulated by inhibition of the MEK–ERK–RSK pathway and is post-transcriptionally down-regulated through the inhibition of the MEK–ERK–non-RSK pathway (Imai et al. 2009). Further studies are needed to accurately elucidate the molecular mechanisms controlling ABCG2 expression.

Wide distribution in normal tissues

Doyle et al. (1998) first examined the expression of ABCG2 mRNA in selected normal human tissues using the *ABCG2* cDNA as a probe in northern blots. Prominent expression was seen in placental tissue and considerably lower levels of expression were found in brain, prostate, small intestine, testis, ovary, colon and liver. Subsequently, an immunohistochemical study with two different ABCG2-specific monoclonal antibodies BXP-21 and BXP-34 detected high expression of ABCG2 in the epithelium of the small intestine and colon, in placental syncytiotrophoblasts, in liver canalicular membranes, in breast ducts and lobules, and in veinous and capillary endothelium (Maliepaard et al. 2001). Using immunohistochemistry and northern blot analyses, Fetsch et al. (2006) observed several additional sites of ABCG2 expression in normal tissues including alveolar pneumocytes, sebaceous glands, transitional epithelium of bladder, interstitial cells of testes, prostate epithelium, endocervical cells and cervical squamous epithelium, small and large intestinal mucosal epithelial cells, pancreatic islet/acinar cells, adrenal zona reticularis, renal cortical tubules and hepatocytes.

Although ABCG2 is widely distributed in normal tissues, its function is not clear. Recent work, relying mainly on the use of *ABCG2*−/− mice, has revealed that the primary biological role of ABCG2 is protecting the organism from a range of xenobiotics (Vlaming et al. 2009).

ABCG2 and stem cells

Conserved expression in stem cells

The side population phenotype, which is characterized by the ability to transport the fluorescent dye Hoechst 33342, has been identified as a characteristic feature of stem cells. Zhou et al. (2001) first proved that ABCG2 was a molecular determinant of the SP phenotype. A number of other studies in a wide variety of organs have also indicated that ABCG2 is responsible for Hoechst 33342 dye efflux pattern and confers the SP cell phenotype both in human and mouse (Kim et al. 2002; Scharenberg et al. 2002; Martin et al. 2004; Jonker et al. 2005). Therefore, ABCG2 has been suggested as a universal marker for various stem cells. Semi-quantitative RT-PCR analyses taken by Zhou et al. (2001) discovered the expression of ABCG2 in SP cells from murine bone marrow, skeletal muscle and cultured embryonic stem cells, as well as in rhesus monkey bone marrow. ABCG2 is also found to be expressed in hematopoietic stem cells (HSCs) from human and zebra fish (Scharenberg et al. 2002; Kobayashi et al. 2008). Recent studies have shown that ABCG2 is expressed in stem cell populations derived from a wide range of tissues, including pancreas (Lechner et al. 2002), lung (Summer et al. 2003), limbal epithelium (Watanabe et al. 2004), heart (Martin et al. 2004), testis (Lassalle et al. 2004), muscle (Meeson et al. 2004), cornea and conjunctiva (Budak et al. 2005), brain (Islam et al. 2005), prostate (Pascal et al. 2007) and embryo (Apati et al. 2008). A summary of ABCG2 expression in stem cells is provided in Table 1. Together, these findings indicate that ABCG2 expression is a conserved feature of stem cells from a wide variety of sources. Therefore, ABCG2 is an attractive candidate marker useful for identifying and isolating stem cells.

Contribution in stem cell proliferation and self-renewal

The conserved expression of ABCG2 in stem cells from various sources suggests that ABCG2 has an important function in stem cell biology. Israeli et al. (2005) put forward that the biological role of ABCG2 in stem cells may be part of the normal tissue regeneration mechanism, probably due to the protection of the small stem cell population from cell death and the preservation of the stem cell homeostasis under extreme stress conditions. Results obtained from the *ABCG2* deficient mouse model suggest that ABCG2 is not essential for normal hematopoietic development (Zhou et al. 2003). However, recent evidence has revealed that ABCG2 plays a crucial role in protecting stem cells.

Zhou et al. (2002) discovered that ABCG2 null hematopoietic cells were significantly more sensitive to mitoxantrone in vivo. This result suggests that the physiological function of ABCG2 expression in HSCs is to provide protection from cytotoxic substrates. Krishnamurthy et al. (2004) reported that ABCG2 enhances the survival of hematopoietic stem cells in hypoxia through its interactions with heme. This can explain why stem cells thrive under conditions of low oxygen. Specifically, ABCG2 binds heme and diminishes the cellular accumulation of porphyins. ABCG2 has also been shown to play a role in protecting embryonic stem cells from porphyrin accumulation during colony expansion (Susanto et al. 2008). In addition, Martin et al. (2008) unveiled a cytoprotective role of ABCG2 in cardiac SP cell populations in response to oxidative stress. Overexpression of ABCG2 leads to the upregulation of cytoprotective factors involved in the oxidative stress response and promotes cellular viability. A recent report by Ahmed et al. (2008) demonstrated that constitutive expression of ABCG2 enhances the proliferative capacity of early human hematopoietic progenitors both in vitro and in vivo. ABCG2 is characterized as a regulatory protein of early human hematopoietic development.

Table 1
Summary of ABCG2 expression in stem cells.

Stem cell types	Major findings
Human pancreatic islet stem cells	Nestin-positive cells derived from human pancreatic islets contain 1.5–2% of SP cells, which express ABCG2 and nestin at high levels compared to non-SP control cells.
Human HSCs	The expression of ABCG2 is restricted to the most immature hematopoietic progenitors in human bone marrow and is sharply down-regulated at the committed progenitor level.
Human limbal epithelial stem cells	Harvested limbal epithelial cells contain SP cells expressing ABCG2.
Mouse germ stem cells	SP phenotype is dependent on ABCG2 activity and testis SP in adult mice is highly enriched in male germ stem cells.
Mouse muscle stem cells	ABCG2 is a determinant of the SP cell phenotype and muscle SP cells are probably progenitor cells that participate in repair and regeneration of adult skeletal muscle.
Mouse heart stem cells	Adult heart contains an ABCG2-expressing SP cell population and these progenitor cells are capable of proliferation and differentiation.
Human and rabbit limbal conjunctival epithelia stem cells	Limbal and conjunctival epithelia contain ABCG2-dependent SP cells.
Human NSPCs	About 63% of the cells in neurospheres were ABCG2-positive and ABCG2 levels were sharply down-regulated during human NSPCs differentiation.
Mouse HSCs	Overexpressed in HSCs.
Human prostate stem cells	The SP transcriptome was essentially the same as ABCG2+ and both populations expressed genes indicative of a stem cell phenotype.
Human ESCs	High level ABCG2 expression in the undifferentiated human ESCs, while the expression of this protein significantly decreased during early cell differentiation.
Zebra fish HSCs	zAbcg2a mRNA is a useful marker for zebra fish HSCs.

HSC: hematopoietic stem cell; NSPC: neural stem/progenitor cell; ESC: embryonic stem cell.
SP: side population.

Taken together, ABCG2 plays a role in protecting stem cells by increasing their survival capacity and proliferation potential, processes which are fundamental for stem cell maintenance and renewal.

Block stem cell differentiation

ABCG2 is sharply down-regulated during hematopoietic stem cell differentiation and is expressed at a low level in mature cells compared with progenitor cells (Zhou et al. 2001; Scharenberg et al. 2002). The same phenomenon also occurs in human neural, retinal and embryonic stem cells (Islam et al. 2005; Bhattacharya et al. 2007; Apati et al. 2008). The highly regulated expression of ABCG2 suggests that ABCG2 may play a regulatory role in maintaining stem cells in an undifferentiated state. When ABCG2 is overexpressed in retinal progenitors by using a retrovirus-mediated transduction, differentiation is blocked, accompanied by an increase in the expression of stem cell markers and the SP cell phenotype. By contrast, siRNA-mediated silencing of ABCG2 expression in retinal progenitors depletes the SP cell population and promotes differentiation (Bhattacharya et al. 2007). In addition, notch signaling, a key regulator of retinal stem cells is shown to influence ABCG2 expression and the SP cell phenotype (Bhattacharya et al. 2007). Therefore, ABCG2 is involved in the maintenance of stem cells under the regulation of notch signaling. Considering that ABCG2 acts as an efflux pump, it is also possible that constitutive expression of ABCG2 in human progenitor cells critically expels substrates that are necessary for lineage differentiation, thereby blocking stem cell differentiation. However, few studies have directly proved this function of ABCG2 in stem cells and more research is required to unveil this phenomenon.

ABCG2 and cancer

ABCG2 expression in human tumors

Diestra et al. (2002) were the first to report on a large screen of ABCG2 expression in human tumors using the monoclonal antibody BXP-21. ABCG2 expression was seen in all 21 tumor types that were studied, with a high frequency in carcinomas of the digestive tract, endometrium, lung and melanoma. Contradictory results of both high and low expressions in acute myelogenous leukemia (AML) and acute lymphocytic leukemia (ALL) have been reported, and controversial data exist regarding the clinical importance of ABCG2 expression in these blood malignancies (Abbott et al. 2002; Steinbach et al. 2002; Sauerbrey et al. 2002; Plasschaert et al. 2003). However, in the largest study reported to date, with 149 AML cases, Benderra et al. (2004) reported that ABCG2 expression was a prognostic factor of complete remission, 4-year disease free survival and 4-year overall survival. In some solid tumors such as ovarian and breast cancer, ABCG2 expression frequently showed no correlation with clinical outcomes (Nakayama et al. 2002; Faneyte et al. 2002). In contrast, ABCG2 expression is detected and generally, this expression is associated with negative prognosis in many other human solid tumors such as lung and esophageal cancers (Tsunoda et al. 2006; Yoh et al. 2004). More recently, ABCG2 expression has been detected in glioma stem cells, and the expression level of ABCG2 correlates well with the increasing pathological grade of glioma, suggesting that ABCG2 may be a marker of melanoma progression (Jin et al. 2009).

Based on a RNA interference approach, Chen et al. (2010) showed that the suppression of ABCG2 could significantly inhibit cancer cell proliferation. Furthermore, the blocking of ABCG2 function by fumitremorgin C, a chemical inhibitor, also inhibited cell proliferation via the prolonged G0/G1 interval. These data suggest that ABCG2 may contribute to cancer cell proliferation.

A recent study evaluated SHH and ABCG2 expression in 67 cases of diffuse large B-cell lymphoma. High levels of ABCG2 in diffuse large B-cell lymphoma tumors correlate with shorter overall survival and failure-free survival and ABCG2 protein levels correlate with the expression of SHH protein levels (Kim et al. 2009). The HH signaling pathway plays a critical role in growth and differentiation during embryonic development (Ingham and McMahon 2001). Aberrant activation of the HH pathway has been shown to contribute to tumor development and progression (Karhadkar et al. 2004; Liao et al. 2009). Because ABCG2 is a downstream target of the SHH pathway, it is possible that the high expression of ABCG2 in tumor cells could be a result of aberrant pathway activity that imparts multiple mechanisms of tumor initiation, growth, invasiveness, metastasis and relapse.

Cancer stem cells

The cancer stem cell hypothesis suggests that the formation and growth of tumors are driven by rare cancer stem cells (Reya et al. 2001). Like stem cells, cancer stem cells possess extensive proliferation and self-renewal capacity. During the last years, CSCs have been identified in human leukemia (Bonnet and Dick 1997) and in diverse solid tumors, including tumors of breast (Al-Hajj et al. 2003), brain (Singh et al. 2004), retinoblastoma (Seigel et al. 2005), melanoma (Fang et al. 2005), liver (Chiba et al. 2006), pancreas (Li et al. 2007), colon (O'Brien et al. 2007), nasopharynx (Wang et al. 2007), lung (Ho et al. 2007), head and neck (Prince et al. 2007), osteosarcoma (Tirino et al. 2008), esophagus (Huang et al. 2009), as well as neuroblastoma (Mahller et al. 2009). Increasing evidence suggests that CSCs play an important role in tumor initiation, progression, metastasis, as well as tumor relapse (Visvader and Lindeman 2008). In order to cure cancer, it is necessary to focus on the elimination of CSCs. To allow a perspective target therapy for cancer, it is important to identify and characterize specific CSC biomarkers.

Biomarkers for CSCs

Two general approaches have been used to identify CSCs. The first one tracks surface markers that identify putative normal stem cells. For example, pancreatic and hepatic CSCs are marked by CD44+ CD24+ESA+ (Li et al. 2007) and CD133+ (Ma et al. 2007), respectively. The second one uses the flow cytometry-based side population technique. Side population cells within tumors have been suggested to be a novel approach to isolate CSCs as it is enriched in normal stem cells. Recent work has shown that SP cells selected from different tumor cell lines and specimens have higher tumorigenicity in immunodeficiency mice, higher colony-forming efficiency and proliferation capacity, compared to non-SP cells (Hirschmann-Jax et al. 2004; Haraguchi et al. 2006; Kruger et al. 2006; Ho et al. 2007; Huang et al. 2009; Zhang et al. 2009). SP cells also show higher chemoresistance to conventional antitumor agents such as doxorubicin and methotrexate when compared with non-SP cells. Together, these findings indicate that SP is enriched with CSCs. However, there are some limitations to this approach. First, the side population does not represent all the cancer stem cells and contains both cancer stem cells and non-cancer stem cells. Moreover, Hoechst 33342, which is used for isolating SP, is toxic to many cells. Thus, the comparison of SP and non-SP is not precise.

ABCG2: a novel target

Taking into account that the SP phenotype is mainly mediated by ABCG2 and the conserved expression of ABCG2 in stem cells, it is conceivable that ABCG2 may serve as a novel biomarker of CSCs. Since ABCG2 functions as a high capacity transporter with a wide range of substrates including various chemotherapy drugs, it has been shown to participate in the multidrug resistance of tumors and lead to a limitation of chemotherapeutics (Robey et al. 2007). Intriguingly, CSCs are also supposed to be responsible for the acquisition of multi-drug chemoresistance and lead to the cancer relapse. Side population

and chemoresistance suggest a close link between ABCG2 and CSCs. ABCG2+ tumor cells may hence represent a unique population of CSCs. The expression of this chemoresistant efflux transporter in CSC populations would confer these cells intrinsic resistance to many commonly used antitumor agents and may be the root cause of tumor recurrence.

Elevated expression of ABCG2 has been observed in a number of putative CSCs from retinoblastoma (Seigel et al. 2005), lung (Ho et al. 2007), liver (Shi et al. 2008) and pancreas cancer (Wang et al. 2009). In addition, ABCG2 and CD133, the widely identified CSC marker, are co-expressed in melanoma and pancreatic carcinoma cell lines (Monzani et al. 2007; Olempska 2007). Recently, Zen et al. (2007) reported ABCG2+ cells could be purified from human hepatocellular carcinoma (HCC) cell lines. ABCG2+ population showed evidence for self-renewal, generating both ABCG2+ and ABCG2− progenies during subculture, and a higher proliferative activity. Moreover, other progenitor cell markers including cytokeratin 19 and alpha-fetoprotein were mainly expressed in ABCG2+ subpopulations. This study suggests that cancer cells with ABCG2 expression might play a central role in hepatocarcinogenesis and the maintenance of the cancer cell hierarchy of human HCC. Our group has already detected high expression of ABCG2 in HCC tissues (Xi et al. 2009) and we are now studying the possible role of ABCG2 in HCC development and progression.

Together, these data suggest that ABCG2 may serve as a promising biomarker for the identification CSCs in tumors. New therapeutic strategies targeting ABCG2+ positive CSCs may effectively eliminate CSCs and overcome current chemotherapeutic limitations.

Conclusion and future perspectives

Because of its extensive expression in stem cells, ABCG2 has gained instant attention from researchers exploring its potential role in stem cell developmental biology. With conserved expression in stem cells, ABCG2 may serve as a universal marker to identify and isolate stem cells from various tissues. In addition, ABCG2 exhibits a special effect in promoting proliferation and blocking differentiation of stem cells which reveals a potential application in stem cell based therapies. Finally, ABCG2 may also have an important biological role in tumors and serve as a potential molecular marker for the further characterization of CSCs. The further characterization of ABCG2 will open up a new avenue in stem cell and tumor therapy.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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