The Stem Cell Marker Bcrp/ABCG2 Enhances Hypoxic Cell Survival through Interactions with Heme

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Received for publication, December 11, 2003, and in revised form, March 1, 2004
Published, JBC Papers in Press, March 24, 2004, DOI 10.1074/jbc.M313599200

Our studies demonstrate that the ABC transporter and marker of stem and progenitor cells known as the breast cancer resistance protein (BCRP or ABCG2) confers a strong survival advantage under hypoxic conditions. We show that, under hypoxia, progenitor cells from Bcrp−/− mice have a reduced ability to form colonies as compared with progenitor cells from Bcrp+/+ mice. Blocking BCRP function in Bcrp−/− progenitor cells markedly reduces survival under hypoxic conditions. However, blocking heme biosynthesis reverses the hypoxic susceptibility of Bcrp−/− progenitor cells, a finding that indicates that heme molecules (i.e. porphyrins) are detrimental to Bcrp−/− cells under hypoxia. BCRP specifically binds heme, and cells lacking BCRP accumulate porphyrins. Finally, Bcrp expression is up-regulated by hypoxia, and we demonstrate that this up-regulation involves the hypoxia-inducible transcription factor complex HIF-1. Collectively, our findings suggest that cells can, upon hypoxic demand, use BCRP to reduce heme or porphyrin accumulation, which can be detrimental to cells. Our findings have implications for the survival of stem cells and tumor cells in hypoxic environments.

Two defining characteristics of stem cells are their ability to transport Hoechst dye and their ability to thrive under conditions of low oxygen (hypoxia) (1–5). Notably, hematopoietic stem cells are concentrated in hypoxic areas, and, under hypoxic conditions, myeloid cells have an improved ability to repopulate the bone marrow of lethally irradiated mice (6). During adaptation to hypoxia, cells express increased amounts of glycolytic enzymes and glucose transporters as they adjust to anaerobic respiration. In addition, heme production within a cell is regulated by environmental oxygen such that heme content increases under hypoxic conditions (7). A central mediator of the cellular response to hypoxia is hypoxia-inducible factor 1 (HIF-1), a basic helix-loop-helix transcription factor and PAS superfamily member. HIF-1 is a heterodimer of α and β subunits, HIF-1α and HIF-1β (also known as the aryl hydrocarbon receptor nuclear translocator or ARNT), and the stability of the α subunit is determined by oxygen concentration (8, 9). The transcription of hypoxia response genes is mediated by HIF-1α, which, in a stable form under hypoxic conditions, binds to HIF-1β. The heterodimer activates transcription by binding to a 5-bp consensus element (RCGTG) known as the hypoxia response element (HRE) (9).

The ability of stem cells to transport Hoechst dye is attributable to the expression of an ABC transporter called the breast cancer resistance protein (BCRP), also known as ABCG2 (ATP-binding cassette, subfamily G, member 2) (1, 2, 10, 11). BCRP is abundantly expressed in various stem cells (hematopoietic, muscle, neural, and testicular) (1) but is not essential for hematopoiesis. BCRP substrate (13). Because pheophorbide a overexpressing cells and inferred that pheophorbide a is a BCRP substrate (13). Because pheophorbide a is structurally similar to protoporphyrin IX (a heme precursor), the skin phototoxicity it caused may have resulted from increased accumulation of cellular porphyrins, which are known photoxins (14, 15). The recent finding that Bcrp-null erythrocytes accumulate protoporphyrin IX supports the theory that BCRP transports porphyrin molecules such as heme (13).

The regulation of porphyrins and heme within a cell is important from two perspectives. First, the accumulation of heme within the cell can ultimately lead to the accumulation of iron and the production of cell-damaging reactive oxygen species by the Fenton reaction (14, 16–18). Second, heme/porphyrin accumulation also leads to the collapse of mitochondrial function.
(19, 20). The regulation of intracellular porphyrin levels is therefore fundamental to cell survival. This regulation is especially important under conditions of low oxygen, when the cellular concentration of heme may increase.

We hypothesized that BCRP expression in stem cells protects hematopoietic stem cells from hypoxic environments by preventing the accumulation of porphyrin that causes mitochondrial death. We therefore investigated the effect of hypoxia on hematopoietic cells from Bcrp-null animals to identify possible regulatory mechanisms and determine the role of BCRP in the survival of hematopoietic stem cells under hypoxic conditions. We found that the hematopoietic cells from Bcrp-null animals had increased sensitivity to hypoxia that was entirely due to the loss of their ability to transport BCRP. Our finding that the loss of their ability to transport BCRP. Our finding that the inhibition of heme biosynthesis rescued the ability of these cells to survive hypoxia indicates that sensitivity to hypoxia is directly linked to heme biosynthesis. Furthermore, we established a link between hypoxic sensitivity and BCRP function by showing that BCRP can bind to heme and that the presence of heme modifies BCRP-mediated transport. Finally, we showed that BCRP is activated via the HIF-1 signaling pathway. Cumulative heme modifies BCRP-mediated transport.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Hepa-1c1c7, BpRcl, Saos-2, JAR, and NIH/3T3 cells were from the American Type Culture Collection (ATCC, Manassas, VA). Hepa-1c1c7 cells were cultured in modified Eagle’s medium without nucleosides supplemented with 10% fetal bovine serum (FBS) and 100 units/ml penicillin. BpRcl cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 4 mM l-glutamine, 1.5 g/l sodium bicarbonate, 100 units/ml penicillin, and 1% FBS. Mammalian cells were engineered to overexpress human BCRP as described previously (21) and cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% FBS, 100 units/ml penicillin, 2 mM l-glutamine, and 500 µg/ml G418. OCI-AML3 (parental) and OCI-AML3/HaABC2 (BCRP-transduced clone 6.2) cells were cultured as described previously (22). Normoxic conditions constituted a humidified atmosphere containing 3% oxygen. Cellular protoporphyrin IX concentration was measured as described previously (23). Briefly, cells were harvested and washed once with PBS, and protoporphyrin IX concentration in the supernatant was determined by using a Vantage flow cytometer (BD Biosciences). To induce protoporphyrin IX fluorescence, the excitation wavelength was set at 405 nm (24), and the emission filter was set at 695 nm/40 nm.

**Transport Studies with Membrane Vesicles**—Sf9 cells were infected with recombinant baculovirus containing the cDNA of human BCRP (wild-type ABCG2). This procedure yielded a high level of human ABCG2 expression (25). Sf9 membrane vesicles were prepared as described previously (26). ATP-dependent uptake of [3H]E35 (167.6 × 10⁻¹⁰ Bq/mmol) was determined by the rapid filtration method (27). Briefly, membrane vesicles containing 12.5 µg of protein were incubated with E35 (10–160 µM) in 50 µl of vesicle buffer (100 mM KCl, 50 mM Hepes, pH 7.4), 4 mM ATP and AMP, 10 mM MgCl₂, 10 mM creatine phosphate, 100 µg/ml creatine kinase, and hemin for 3 min at 37 °C. The reaction mixture was then diluted in 2 ml of ice-cold PBS and filtered immediately through a cellulose filter (0.45-µm pore size). The filters were washed twice with 2 ml of ice-cold PBS, and the radioactivity retained on the filters was measured by liquid scintillation. Uptake was defined as the difference between the non-specific binding of the substrate to vesicles at 4 °C and the total substrate associated with the vesicles at 37 °C. ATP-dependent transport was evaluated by calculating the difference between uptake in the presence of ATP and uptake in the absence of ATP in membrane vesicles prepared from Sf9 cells expressing BCRP or transfected with vector only.

**Cell Viability Studies**—We seeded JAR or OCI-AML3 cells into 60-mm culture dishes (2 × 10⁵ cells per dish) on day 0 and imposed normoxia or hypoxia for 24 h starting on day 2. Then, after a 1-h incubation with Hoechst 33342 (Molecular Probes, Eugene, Oregon) at a final concentration of 0.5 µg/ml, we washed the cells in ice-cold PBS, trypsinized them, and resuspended them in ice-cold PBS. To sort the cells we used a Vantage flow cytometer (BD Biosciences) with an excitation wavelength of 350 nm and detection wavelengths of 450 (blue) and 480 (green) nm. A live gate was defined to exclude dead cells.

**Cytotoxicity Assay**—In 96-well plates we incubated JAR cells (~10⁵ cells per well) and OCI-AML3 cells (~10⁵ cells per well) at 37 °C for 24 h and then added SN-38 (kindly provided by Dr. Clinton Stewart, St. Jude Children’s Research Hospital, Memphis, Tennessee) in a dilution series and continued the incubation for 3–4 days. Reserpine, when included (~1 µM), was added on day 4 of the assay. Months of storage delayed the onset of resistance, but the effect was not due to the absence of drug. Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide assay (28).

**Side Population (SP) Cell Analysis—**Bone marrow cells from Bcrp⁻/⁻ and Bcrp⁺/⁺ mice were isolated as described above and exposed to air or hypoxia (0.1–0.5% oxygen). SP cell analysis was done as described elsewhere (21).
Construct. Transfected cells were subjected to hypoxia or normoxia for 24 h, and luciferase activity was assessed in total lysates by using an Opticon luminescence reader as described previously (31). In a subset of experiments, we introduced HIF-1 binding site mutations into the Bcrp promoter by using a site-directed mutagenesis kit (Stratagene). Briefly, three-nucleotide mutations (consensus motifs 5'-GCGCTTT-3') were introduced into the HIF-1 binding site of the Bcrp gene, and their KSL phenotype was determined after subtraction of the values obtained for pGL3-luciferase, and the results were expressed in relative terms (ratio of hypoxic value to normoxic value). We performed all experiments in duplicate at least three times. For cotransfections, cells were cotransfected with 1 µg each of the pGL3-Bcrp promoter constructs or a pGL3 vector and 0, 2, or 4 µg of pcDNA3-HIF-1α or pcDNA3 vector alone. We used LipofectAMINE transfection reagent (Invitrogen) according to the manufacturer’s instructions and kept the total amount of DNA added to each well constant.

Electrophoretic Mobility Shift Assays—We translated recombinant HIF-1α and HIF-1β proteins in vitro by using a transcription and translation kit (TNT, Promega). We prepared a double-stranded 32P-labeled oligonucleotide that represents the HRE of the Bcrp promoter (underlined) at base pairs −116 to −112 (5’-GCAGGACACGTGT-GCCCTTT-3’) and contains the corresponding nucleotides −123 to −104 of the Bcrp promoter (32). A portion of the recombinant HIF-1α and HIF-1β were prereacted (10 min) with an unlabeled specific probe (that contains the HRE shown above in a 10-, 50-, or 250-fold molar excess) or an unlabeled nonspecific double-stranded oligonucleotide (corresponding to bases −362 to −342 of the Bcrp promoter in a 250-fold molar excess) in 15 µl of reaction buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 4.0% glycerol, 0.5 mM EDTA, 1.0 mM MgCl2, 0.5 mM dithiothreitol, and 0.75 µg of poly(dI-dC) (Promega)). The mixture was then incubated with 0.046 pmol of the 32P-labeled probe (20 min). Complexes were resolved on a nondenaturing 6% polyacrylamide gel (Invitrogen) and detected by autoradiography.

RESULTS

Bcrp-mediated Transport Protects Cells from Death under Hypoxic Conditions—To test the role of Bcrp in the survival of hematopoietic progenitor cells under hypoxic conditions, we purified primitive hematopoietic progenitor cells (identified by their KSL phenotype) from Bcrp+/− mice and their Bcrp+/+ litter mates. We evaluated the survival of these cells under hypoxia (3% O2) and normoxia (20% O2) in a 250-fold molar excess) in 15 µl of reaction buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 4.0% glycerol, 0.5 mM EDTA, 1.0 mM MgCl2, 0.5 mM dithiothreitol, and 0.75 µg of poly(dI-dC) (Promega)). The mixture was then incubated with 0.046 pmol of the 32P-labeled probe (20 min). Complexes were resolved on a nondenaturing 6% polyacrylamide gel (Invitrogen) and detected by autoradiography.
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Fig. 2. The stem cell population increases in bone marrow cells exposed to hypoxia, and Bcrp expression increases in cell cultures exposed to hypoxia. When exposed to hypoxia, bone marrow cells from Bcrp+/− mice show an increased proportion of SP cells (A), whereas bone marrow cells from Bcrp−/− mice (B) show a negligible change in SP cells. C, the result of RT-PCR analysis showing expression of a BCRP mRNA transcript in three human cell lines grown under normoxic (−) or hypoxic (±) conditions. D, Hoechst dye efflux characteristics of JAR cells and OCI-AML3 cells under normoxic and hypoxic conditions. Inhibition of hypoxia-induced BCRP activity with reserpine results in reduced efflux. Data are presented as the amount of Hoechst dye efflux under hypoxia relative to that under normoxia.

animals were exposed to mitoxantrone (1 ng/ml) and normoxia, the colony survival rate (i.e. the number of colonies formed by the mitoxantrone-exposed cells as a percentage of that formed by control cells not exposed to mitoxantrone) was 40%. However, Bcrp+/− progenitor cells exposed to mitoxantrone and normoxia formed no colonies at any of the doses tested (Fig. 1A). When exposed to hypoxic conditions (3% O2), progenitor cells from Bcrp+/− animals showed unimpaired colony-forming ability, but those from Bcrp−/− animals formed half as many colonies as they did under normoxia (Fig. 1B).

Incubation of Bcrp+/− progenitor cells under hypoxia with a BCRP inhibitor, fumitremorgin C (34) or reserpine (1, 35), reduced the colony-forming ability of these cells to a level similar to that of the Bcrp−/− cells (Fig. 1A, B and C). Similarly, the survival advantage conferred by BCRP overexpression in human myeloid cells (OCI-AML3, Fig. 1D) (22) incubated under hypoxic conditions was lost by inclusion of the BCRP inhibitor reserpine (Fig. 1E). Blocking BCRP transport activity therefore rendered the BCRP-overexpressing cells as sensitive to hypoxia as were the control cells. This set of results indicates that BCRP protects cells from death under hypoxic conditions and that it is the transport activity of BCRP that confers this protective effect.

Increased Expression of Bcrp Correlates with Increased Transport Capacity in Multiple Cell Types—Because Bcrp−/− cells showed diminished survival rates (44%, Fig. 1B, and 21%, Fig. 1C) that may be attributable to the different oxygen tensions at which the cells were evaluated (3% for those with a 44% survival rate and 0.1% for those with a 21% survival rate), we investigated Bcrp expression in relation to hypoxia. First, we tested the ability of bone marrow cells from Bcrp+/− and Bcrp−/− animals to efflux Hoechst 33342 in relation to the proportion of SP cells in the sample. SP cells are commonly thought to represent the stem cell component of a population (1). We determined the relative proportions of SP cells present after exposure to hypoxia and normoxia and found that the hypoxic Bcrp+/− cultures produced a 24-fold higher proportion of SP cells than did the normoxic cultures (Fig. 2A). In contrast, the hypoxic Bcrp−/− cultures produced only a 2-fold higher proportion of SP cells than did their normoxic counterparts (Fig. 2B). The large proportion of SP cells in Bcrp+/− bone marrow is reflected in a high level of expression of Bcrp mRNA in the bone marrow of the Bcrp+/− mice (not shown). The much larger proportion of SP cells in the Bcrp−/− cultures subjected to hypoxia indicates that BCRP is responsible for most of the increase in Hoechst dye efflux induced by hypoxia.

To verify this finding in other cell types, we exposed cells of several human lineages (placental/choriocarcinoma (JAR) cells, osteosarcoma (Saos-2) cells, and myeloid leukemia cells (OCI-AML3 cell line)) to hypoxia and then used RT-PCR to analyze BCRP mRNA expression in the cells. EPO, one gene activated by hypoxia, was used as a positive control (Fig. 2C). Typical of cells of placental origin (32, 36), the JAR cells had detectable basal levels of BCRP mRNA, and hypoxia resulted in BCRP up-regulation. BCRP mRNA was undetectable in Saos-2 and OCI-AML3 cells under normoxia but was strongly induced under hypoxia (Fig. 2C). We also tested whether hypoxia-induced increased BCRP activity by evaluating Hoechst 33342 efflux after normoxia or hypoxia (Fig. 2D). Reserpine was used to inhibit BCRP-mediated Hoechst 33342 efflux to determine how much Hoechst efflux was attributable to BCRP activity (1). Hypoxia increased the proportion of cells that expelled Hoechst 33342 by 7-fold, a value that corresponds to the increase in mRNA (Fig. 2, C and D). Notably, in the presence of reserpine, cells cultured under hypoxic conditions lost their enhanced ability to efflux Hoechst dye and reverted to their normoxic phenotype (Fig. 2D). These studies indicate that hypoxia increases the capacity of cells of various lineages to efflux Hoechst dye and that this increased capacity is correlated with the increased expression of BCRP mRNA in primary murine bone marrow cells and in human cell lines of diverse lineages.

HIF-1 Activates Transcription of BCRP by Binding the HRE Site at Base Pair −115—To test the involvement of the HIF-1 pathway in the hypoxia-induced up-regulation of murine Bcrp,
we examined the responses to hypoxia of a pair of murine cell lines, one with fully functioning HIF-1 (Hepa-1c1c7 cells) and one deficient in HIF-1 (BpRcl cells). HIF-1β is the heterodimeric partner of HIF-1α that is required for DNA binding and transcription of hypoxia response genes. HIF-1-responsive genes are not activated in the HIF-1β-deficient BpRcl cells.
Hypoxia induces a marked increase in the expression of Bcrp mRNA in Hepa-1c1c7 cells (37, 40), but hypoxia had no effect on Bcrp expression in our BpRcl cells (Fig. 3A). We used a computer algorithm (Transfac) to analyze the 5′-flanking sequence of the human gene encoding BCRP and found putative HREs (RCGTG) at three locations, −1059 to −1055 bp, −194 to −190 bp, and −116 to −112 bp, all upstream of the transcription start site (32). To assess the contribution of HREs to BCRP promoter transactivation by hypoxic stimuli, we transfected cells with BCRP promoter constructs encoding deletions of each of the three putative HREs. Each construct was fused to a luciferase reporter. Using the EPO promoter as a positive control, we evaluated the effect of hypoxia on the transcriptional activation of the BCRP promoter. The most distal HRE (−1059 to −1055 bp) was not required for transcriptional activation of the BCRP promoter by hypoxic stimuli (not shown).

To assess the relative contributions of the two proximal putative HREs of the Bcrp promoter that are contained in the −312 to +362-bp promoter construct (Fig. 3B), we performed site-directed mutagenesis and cotransfected cells with these Bcrp promoter constructs and various amounts of an HIF-1α expression vector. HIF-1α activated the −312 to +362 bp BCRP promoter construct in a dose-dependent manner (Fig. 3B). Mutation of the HRE at base pair −194 (construct M2) slightly diminished the HIF-1α activation, but mutation of the HRE at the downstream base pair, −116 (construct M3), substantially reduced HIF-1 activation, as did mutation at both HRE sites (construct M2M3, Fig. 3B). These results support the conclusion that the proximal HRE at base pair −116 is the main HIF-1α binding site in the BCRP promoter. This conclusion was confirmed by an electrophoretic mobility shift assay with oligonucleotide probes to the HREs at −194 and −116 bp (Fig. 3C). The HRE at −116 bp bound strongly to the HIF-1 complex (HIF-1α and HIF-1β), whereas the −194 bp construct bound only weakly to the recombinant HIF-1 complex (not shown). These findings indicate that the BCRP promoter is directly activated by HIF-1 and that the HRE at −116 bp is essential for the transcriptional activation of the Bcrp promoter.

Inhibition of Heme Biosynthesis Enhances Survival under Hypoxic Conditions—The finding that Bcrp-null erythrocytes accumulate protoporphyrin IX suggests that BCRP transports porphyrin molecules, an example of which is heme (13). To test this hypothesis, we determined the amount of protoporphyrin IX in bone marrow cells from Bcrp+/– and Bcrp+/- animals. The bone marrow cells from Bcrp+/- animals contained a greater amount of protoporphyrin IX than did those from Bcrp+/- animals (Fig. 4A). Furthermore, Bcrp-overexpressing cells accumulated a smaller amount of protoporphyrin IX than did control cells (Fig. 4B). Because excess porphyrins cause
mitochondrial death and excess heme generates reactive oxygen species that are deleterious to multiple cell lineages (14, 41, 42), we tested whether BCRP protects cells from excess porphyrin accumulation during hypoxia by evaluating progenitor cell survival under conditions in which heme biosynthesis was inhibited by succinyl acetone. Succinyl acetone is a potent inhibitor of the heme biosynthetic enzyme aminolevulinate dehydratase and, as expected, leads to decreased levels of protoporphyrin IX in bone marrow cells treated with succinyl acetone (included as supplemental material in the on-line version of this article) (43, 44) (Fig. 4C). Under normoxic conditions, succinyl acetone did not affect the survival of Bcrp−/− and Bcrp+/+ progenitors (data not shown). Under hypoxic conditions, progenitor cells from Bcrp−/− mice had a remarkably high survival rate in the presence of succinyl acetone (Fig. 4C). Interestingly, Bcrp−/− cells also had an increased survival rate in the presence of succinyl acetone. The finding that survival is enhanced under hypoxic conditions when heme biosynthesis is inhibited supports the theory that the accumulation of heme is the predominant cause of cell death under hypoxic conditions and that BCRP transports porphyrins. Moreover, the level of HIF-α is almost identical in the Bcrp−/+ versus Bcrp−/− bone marrow, indicating that altered Hif-1 levels do not account for the difference in response (included as supplemental material in the on-line version of this article).

The rate-limiting enzyme in heme biosynthesis, 5-aminolevulinate synthetase (ALA-s), is up-regulated by hypoxia (7). We therefore evaluated the expression of ALA-s in Bcrp−/− and Bcrp+/+ bone marrow cells (Fig. 4D). The basal level of ALA-s mRNA was slightly higher in Bcrp−/− than in Bcrp+/+ bone marrow cells, and hypoxia induced ALA-s expression to a greater extent in Bcrp−/− than in Bcrp+/+ bone marrow cells. This hypoxia-induced increase in expression of the rate-limiting enzyme in heme biosynthesis might lead to higher porphyrin levels in Bcrp−/− cells (Fig. 4A), which are cytotoxic, and could explain why treatment with succinyl acetone resulted in a high survival rate of Bcrp−/− cells under hypoxic conditions (Fig. 4C). This protective effect of succinyl acetone appears, therefore, to be a consequence of its ability to prevent heme biosynthesis and thus avoid accumulation of cellular porphyrins.

BCRP Interacts Specifically with Heme—We further examined the role of BCRP in controlling heme or porphyrin accumulation by exploring the association between the transporter and its substrate, i.e. the association between BCRP and the end product of porphyrin biosynthesis, heme. We prepared crude plasma membranes containing BCRP from cells engineered to express human BCRP and tested the ability of the affinity resin, hemin-agarose, to precipitate the plasma membranes by binding to the BCRP in them (Fig. 5A). The physical interaction between hemin-agarose and BCRP was dose-dependently inhibited by hemin (Fig. 5B). To test whether the interaction of BCRP with hemin-agarose was primarily ionic, we varied the amount of sodium ion present. The hemin:BCRP interaction was disrupted at a sodium concentration above 400 mM (Fig. 5C). This finding indicates that ionic interactions play an important role in the binding of heme to BCRP.

To determine whether the binding of heme to BCRP is altered in the presence of BCRP substrates, we repeated the hemin-agarose BCRP precipitation assay (above, and in Fig. 5A) in the presence of various concentrations of BCRP substrate, mitoxantrone, or Hoechst 33342 (45–48). The amount of BCRP precipitated by hemin-agarose dose-dependently increased in the presence of each substrate (Fig. 5, D and E). Moreover, as the concentration of mitoxantrone or Hoechst 33342 was increased, the amount of BCRP precipitated increased until BCRP was no longer detectable in the supernatant. This finding indicates that these drug substrates enhanced the avidity of hemin-agarose for BCRP. Notably, and consistent with the suggestion that Hoechst 33342 is a better BCRP substrate than mitoxantrone (35, 49), hemin-agarose more readily precipitated BCRP in the presence of Hoechst 33342 than in the presence of mitoxantrone.

To characterize the relation between BCRP transport and heme, we examined the effect of heme on the transport of E3S, another BCRP substrate (50), by using membrane vesicles prepared from insect cells programmed to express wild-type human BCRP (49). As the concentration of heme increased, the velocity of E3S transport increased (Fig. 5F); this finding supports the theory that BCRP and heme interact cooperatively. Indeed, cooperative interactions between transporter and substrate have been noted for other ABC transporters (e.g. multidrug resistance protein 2) and interpreted as the co-transport of substrate molecules (27, 51). Overall, our findings indicate that BCRP interacts specifically with heme for the following reasons: 1) Hemin dose-dependently prevented BCRP from interacting with hemin-agarose (Fig. 5B). 2) The association of BCRP with heme occurred through ionic interactions and was prevented at elevated salt concentrations (Fig. 5C). 3) BCRP substrates modified the interaction of heme with BCRP (Fig. 5, D and E); and 4) heme modified BCRP transport activity (Fig. 5F).

DISCUSSION

Our findings indicate that BCRP has a physiological role in the survival of hematopoietic stem cells under hypoxic conditions. By using chemical inhibitors of BCRP transport and by examining Bcrp-null cells, we have established that BCRP protects cells from hypoxia and that the magnitude of this
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*J. Biol. Chem.* 2004, 279:24218-24225.
doi: 10.1074/jbc.M313599200 originally published online March 24, 2004

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