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Fanconi anemia genes are highly expressed in primitive CD34⁺ hematopoietic cells

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Abstract

Background: Fanconi anemia (FA) is a complex recessive genetic disease characterized by progressive bone marrow failure (BM) and a predisposition to cancer. We have previously shown using the *Fancc* mouse model that the progressive BM failure results from a hematopoietic stem cell defect suggesting that function of the FA genes may reside in primitive hematopoietic stem cells.

Methods: Since genes involved in stem cell differentiation and/or maintenance are usually regulated at the transcription level, we used a semiquantitative RT-PCR method to evaluate FA gene transcript levels in purified hematopoietic stem cells.

Results: We show that most FA genes are highly expressed in primitive CD34-positive and negative cells compared to lower levels in more differentiated cells. However, in CD34⁺ stem cells the *Fancc* gene was found to be expressed at low levels while *Fanccg* was undetectable in this population. Furthermore, *Fanccg* expression is significantly decreased in *Fancc*^{-/-} stem cells as compared to wild-type cells while the cancer susceptibility genes *Brca1* and *Fancc1/Brca2* are upregulated in *Fancc*^{-/-} hematopoietic cells.

Conclusions: These results suggest that FA genes are regulated at the mRNA level, that increased *Fancc* expression in LTS-CD34⁺ cells correlates with a role at the CD34⁺ differentiation stage and that lack of *Fancc* affects the expression of other FA gene, more specifically *Fanccg* and *Fancc1/Brca2*, through an unknown mechanism.

Background

Fanconi anemia (FA) is a genetic autosomal recessive disease characterised by bone marrow (BM) failure associated with cancer susceptibility and congenital defects [1]. Somatic cell fusion studies of FA cells have revealed eight complementation groups suggesting the existence of at least eight genes implicated in the disease [2]. Six FA genes have been cloned, *FANCA*, *FANCC*, *FANCD2*, *FANCE*,

FANCF and *FANCG* and recently the Breast cancer susceptibility gene, *BRCA2*, has been assigned to complementation group D1 and possibly to group B although this requires further confirmation [3–10]. Knockout mouse models from complementation group A (*Fanca*^{-/-}), C (*Fancc*^{-/-}) and G (*Fanccg*^{-/-}) have been generated in order to study the molecular basis of this disease [11–14]. Bone marrow (BM) failure similar to that observed in FA

patients was shown in *Fancc*^{-/-} mice after treatment with a DNA damaging agent, mitomycin C, showing progressive pancytopenia and reduced BM cellularity [15]. Also, hematopoietic stem cells from *Fancc*^{-/-} mice were shown to have a reduced long-term reconstitution ability in recipient mice concomitant with a reduced number of primitive (Lin^-) $\text{Thy1.2}^{\text{lowc-kit}^+ \text{Sca-1}^+ \text{CD34}^+$ hematopoietic cells [16,17]. These results suggest that FA genes may be involved at specific stages of stem cell growth and/or differentiation. Since genes involved in stem cell differentiation are highly regulated at the RNA level [18–22], we evaluated FA genes expression pattern in murine hematopoietic cell populations more specifically in lineage-negative (Lin^-) $\text{Thy1.2-Sca-1}^+ \text{CD34}^+$ and $\text{Thy1.2-Sca-1}^+ \text{CD34}^-$ stem cells. Our results show that in wild-type BM, FA genes are highly expressed in the $\text{Lin}^- \text{Thy1.2-Sca-1}^+ \text{CD34}^+$ stem cell population and that *Fancc* gene expression is altered in *Fancc*^{-/-} hematopoietic cells.

Methods

Hematopoietic cell purification

Bone marrow (BM) cells were obtained from 4 to 6 month old *Fancc*^{-/-} and *Fancc*^{+/+} mice (C57BL/6J, 11th generation of backcrosses) as previously described [17]. Bone marrow were collected from femurs, tibias and humeri, resuspended in PBS supplemented with 2% fetal bovine serum (FBS, Life Technologies, Burlington, ON, Canada) and depleted of red blood cells in ammonium chloride solution (StemCell Technologies Inc., Vancouver, BC, Canada) for 10 min at 4°C. Total BM cells were either used for RT-PCR analysis or further divided into subpopulations as follows. Total BM cells were depleted of lineage (CD5, CD45R, CD11b, myeloid differentiation antigen Gr-1, TER 119) and Thy1.2 positive cells using StemSep negative cell selection procedure as described by the manufacturer (StemCell Technologies Inc., Vancouver, BC, Canada). The lineage and Thy1.2 depleted (Lin^-) cell population obtained was either used for gene expression analysis or further sorted into $\text{Sca-1}^+ \text{CD34}^+$ (LTS- CD34^+) or $\text{Sca-1}^+ \text{CD34}^-$ (LTS- CD34^-) populations as previously described [17]. Briefly, $\text{Lin}^- \text{Thy1.2}^-$ cells were labeled with fluorochrome conjugated rat anti-mouse antibodies directed against CD34 and Sca-1 antigens (FITC-CD34 and PE-Sca-1, Ly-6A/E; PharMingen, Mississauga, ON, Canada). Rat IgG2a monoclonal antibodies conjugated to FITC or PE were used as isotype standards. Cells were labeled 30 min at 4°C, washed and resuspended in PBS supplemented with 2% FBS. Cells were sorted on an Epics Coulter cell sorter (BeckmanCoulter Canada, Mississauga, ON, Canada). Cells were defined by forward (FSC) and side (SSC) scatter and gated for Sca-1^+ and CD34^+ (LTS- CD34^+) or CD34^- (LTS- CD34^-) populations (Figure 1A). Re-analysis of sorted $\text{Sca-1}^+ \text{CD34}^+$ and $\text{Sca-1}^+ \text{CD34}^-$ cells showed a purity of 85 to 95% in each fraction (Figure 1B). Purity of each cell fraction was also

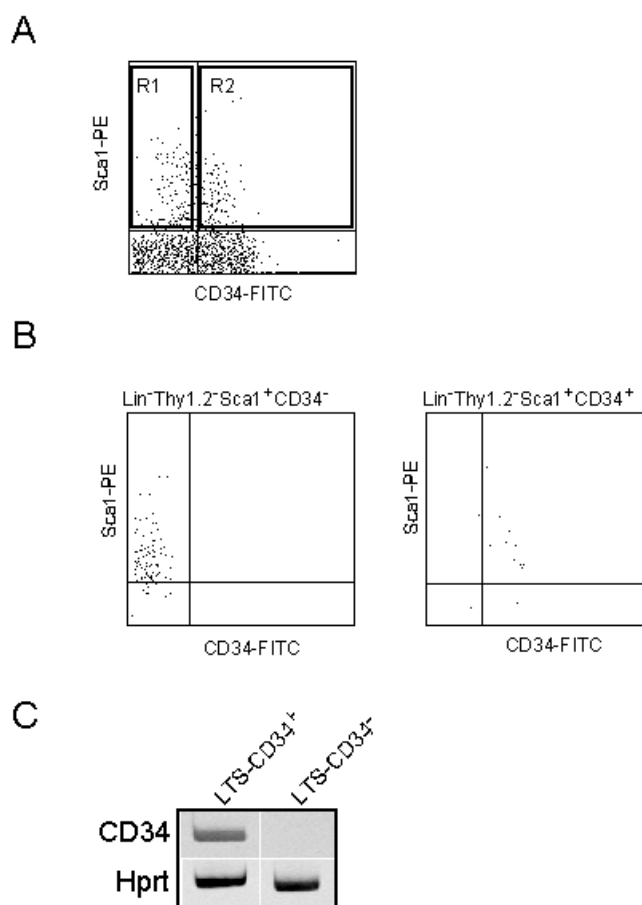


Figure 1
Sorting regions used for LTS- CD34^+ and LTS- CD34^- cell purification. (A) Lineage and Thy1.2 positive cells were removed from murine BM cell preparation as described in materials and methods. $\text{Lin}^- \text{Thy1.2}^-$ cells were sorted on the basis of CD34 and Sca-1 expression using R1 and R2 sorting windows. (B) Reanalysis of sorted $\text{Lin}^- \text{Thy1.2}^- \text{Sca1}^+ \text{CD34}^-$ and $\text{Lin}^- \text{Thy1.2}^- \text{Sca1}^+ \text{CD34}^+$ cells. (C) Representative gel electrophoresis of RT-PCR products in sorted $\text{Lin}^- \text{Thy1.2}^- \text{Sca1}^+ \text{CD34}^-$ and $\text{Lin}^- \text{Thy1.2}^- \text{Sca1}^+ \text{CD34}^+$ cells from wild-type mice using either murine CD34 or Hprt primers.

evaluated by RT-PCR as described below using specific murine CD34 primers (Table 1). No CD34 mRNA expression was detected in sorted LTS- CD34^- fraction (Figure 1C). Animal experiments were approved by the Animal Care Committee of Laval University, QC, Canada.

RNA extraction and RT-PCR analysis

One to 5×10^6 of total hematopoietic or $\text{Lin}^- \text{Thy1.2}^-$ cells were used for total RNA purification and RT-PCR analysis. Total RNA was extracted using TRIZOL reagent (Life technologies) as described by the manufacturer and

Table 1: Oligonucleotides primers used for PCR analysis

Genes	Primer sequences	Position on cDNA	Size of PCR fragment	Accession number
<i>Fanca</i>	S 5'-tccttgtcagcgagatcatg	2955	457	XM125090
	A 5'-cctgaagaagtgatggtaat	3412		
<i>Fancc</i>	S 5'-cttacgggtctccatgtcttg	1337	653	NM007985
	A 5'-ctgagcagcatcaggagacgg	1990		
<i>Fancd1/Brca2</i>	S 5'-agcccatgacagcctccacttg	9394	259	NM009765
	A 5'-cggggacggtaagggcagcc	9653		
<i>Fancd2</i>	S 5'-gccgggctttgagatgatc	913	418	AK019136
	A 5'-ccgtgcaggaccagaaca	1331		
<i>Fance</i>	S 5'-ggcctctgctcctctctgtg	1103	246	AK013325
	A 5'-gctctgctgctctgtgatctg	1349		
<i>Fancf</i>	S 5'-cacgaggtccctacacagatggaggacatg	ND	228	*H.Joenje
	A 5'-agcctgggaactgagaatctactctagcac			
<i>Fang</i>	S 5'-tccccacccacctctctctag	1176	317	AY049715
	A 5'-caggcagggtccgaaagagcag	1493		
<i>Brca1</i>	S 5'-cagcggggcaccatgaagtataa	4260	304	XM126697
	A 5'-ggccttcccctcctgactcgt	4564		
<i>Hprt</i>	S 5'-agtcccagcgtcgtgattag	100	269	NM013556
	A 5'-aggaatggatctactactat	369		
CD34	S 5'-atgcagggtccacagggacacg	51	220	BC006607
	A 5'-ctgtcctgatgatcaagtag	271		

S, sense; A, anti-sense; ND, unknown *Primer sequences provided by Dr. H.Joenje, Free University Amsterdam

resuspended in diethylpyrocarbonate (DEPC)-treated water containing ribonuclease inhibitor Superase[•] in[™] (1 U/ μ l, Ambion, Austin, TX, USA). For total BM and Lin⁻Thy1.2⁻ cells, RNA concentration was quantified by spectrophotometry at 260 nm and 400 ng of total RNA was used for RT-PCR analysis. For LTS-CD34⁺ and LTS-CD34⁻ stem cell populations, the RNA extraction protocol was modified to improve yield from small cell numbers ranging from 5000 to 35000 cells. Briefly, total RNA was extracted using TRIZOL reagent and precipitated with glycogen as carrier. Each RNA preparation from LTS-CD34⁺ and LTS-CD34⁻ stem cells was used directly in RT-PCR reactions. The reverse transcription reaction was done with oligo (dT) primers using the SuperScript[™]II protocol as described by the manufacturer (Life technologies). For PCR amplification, 10 to 20 % of cDNAs from each cell preparation was directly added to a standard PCR amplification mixture. Sense and anti-sense primer sequences and the corresponding cDNA PCR product sizes are shown in Table 1. First, we determined optimal PCR conditions for linear amplification of gene transcripts and to avoid saturation conditions. Linear amplification of the hypoxanthine guanine phosphoribosyl transferase gene (*Hprt*) were obtained with cDNA obtained from 10 to 50 ng of total RNA or with RNA extracted from 6 000 to 60 000 hematopoietic cells (data not shown). Thirty cycles for BM or Lin⁻ cell RNA extract or 35 cycles for LTS-CD34⁺ or LTS-CD34⁻ cell RNA extract was carried out and found to be in the linear PCR reaction range. PCR products were separated on 8% polyacrylamide gel and visualized by

ethidium bromide staining. Volumes of 5 or 10 μ l of PCR products were deposited on gels according to the expression level of each gene. Signal intensities were analyzed using the BioRad Quantity one[™] software. The relative expression level for each target gene was quantified by calculating the ratio of the target gene intensity signal to the *Hprt* intensity signal obtained from the same cDNA preparation and visualized on the same gel. Specificity of all primers was further confirmed by direct nucleotide sequencing analysis of PCR products. Each RNA extraction products were tested and found free of DNA contamination using PCR amplification on samples without RT reactions. Statistical analysis were performed using the Student *t*-test.

Results

FA genes are highly expressed in murine wild-type primitive hematopoietic cells

Several studies have surveyed changes in the expression profiles of many genes associated with hematopoietic cell growth and differentiation such as transcription factors, cytokine receptors and ligands, cell cycle related genes and integrins [18–22]. Since the hematopoietic defect in Fanconi anemia is thought to reside in the stem cell compartment [17], we sought to determine the expression profile of FA genes in murine hematopoietic stem and progenitor cells. We evaluated the mRNA expression levels of FA genes from complementation group A, C, D1/Brca2, D2, E, F and G in sorted hematopoietic stem cell fractions using a semiquantitative RT-PCR procedure. The relative

Table 2: FA gene expression ratios in *Fancc*^{-/-} hematopoietic cell population as compared to *Fancc*^{+/+} cells

	BM	Lin ⁻ Thy1.2 ⁻	LTS-CD34 ⁺	LTS-CD34 ⁻
<i>Fanca</i>	5.1 ↑	7.0 ↑	0.45	1.3
<i>Fancd1/Brca2</i>	3.3 ↑	2.6 ↑	2.9	0.58
<i>Fancd2</i>	4.3 ↑	0.18 ↓	0.76	1.5
<i>Fance</i>	0.75 ↓	0.95	1.5	0.37
<i>Fancf</i>	1.1	1.1	0.46	0.79
<i>Fancg</i>	1.8	1.3	0.06 ↓	-
<i>Brca1</i>	7.0 ↑	2.5 ↑	1.7	1.7

↑ Significant increased expression levels in *Fancc*^{-/-} cells; ↓ Significant decreased expression levels in *Fancc*^{-/-} cells -: Gene expression not detected neither in *Fancc*^{+/+} nor *Fancc*^{-/-} cells

expression of each FA gene was based on the housekeeping gene *Hprt* transcription level (equivalent to 1) in each sample tested allowing the comparison of *Fanc* gene expression levels between murine BM, lineage and Thy1.2-depleted, LTS-CD34⁺ and LTS-CD34⁻ hematopoietic cells. We found that *Fanca*, *Fancc*, *Fancd2* and *Fancg* were highly expressed in LTS-CD34⁺ cells (*Fanc/Hprt* ratios ranging from 0.45 to 0.75) whereas *Fancd1/Brca2*, *Fance*, *Fancf* and the FA-associated cancer susceptibility gene *Brca1* were expressed at low levels in these cells with ratios ranging from 0.09 to 0.30 (Figure 2 and 4). Expression of all *Fanc* genes tested was dramatically low in both BM and Lin⁻Thy1.2⁻ cells with relative expression ranging from 0.029 to 0.36. Both *Fance* and *Fancf* were expressed at low levels in all BM cell populations tested with relative expression of 0.07 to 0.27 and 0.14 to 0.30 respectively.

Adult murine long-term repopulating stem cells are thought to reside in the CD34⁻ cell compartment while CD34⁺ cells have short-term reconstitution potential [23–25]. We measured *Fanc* gene mRNA expression in the LTS-CD34⁻ cell fraction. Our results show that *Fanca* is highly expressed in LTS-CD34⁻ cells (ratio of 0.875 ± 0.045) whereas other *Fanc* genes showed low mRNA expression levels (ratio ranging from 0.085 to 0.33). In addition, we were unable to detect *Fancg* expression in LTS-CD34⁻ stem cells (4 independent reactions). Our results suggest that most *Fanc* genes, and more dramatically *Fancc* and *Fancg* are upregulated in murine LTS-CD34⁺ cells correlating with a specific role at this hematopoietic cell differentiation stage.

Fanc genes are downregulated in *Fancc*^{-/-} hematopoietic cells

We investigated the expression levels of *Fanc* genes in *Fancc*^{-/-} hematopoietic cells in order to verify if absence of one FA gene, notably the murine *Fancc*, affects the expression profile of other *Fanc* genes. Our results show that *Fanca*, and *Fancd2* are significantly upregulated in *Fancc*^{-/-} total BM cells as compared to *Fancc*^{+/+} cells while *Fance*, *Fancf* and *Fancg* expression levels are either down

regulated or unchanged (Figure 3). Similarly, *Fanca* showed a 7 fold significant increase in mRNA expression in *Fancc*^{-/-} progenitors (Lin⁻Thy1.2⁻) compared to *Fancc*^{+/+} cells (Table 2). Surprisingly, *Fancc*^{-/-} progenitor cells showed lower *Fancd2* mRNA levels with an expression ratio of 0.18 compared to *Fancc*^{+/+} cells while *Fance*, *Fancf* and *Fancg* expression levels were similar between *Fancc*^{-/-} and *Fancc*^{+/+} Lin⁻Thy1.2⁻ cells.

We also evaluated *Fanc* mRNA expression in both CD34⁺ and CD34⁻ stem cells. We show that *Fancg* expression is significantly decreased in *Fancc*^{-/-} LTS-CD34⁺ cells as compared to *Fancc*^{+/+} cells (ratio of 0.06). Again, *Fancg* expression could not be detected in *Fancc*^{-/-} LTS-CD34⁻ cells as in *Fancc*^{+/+} cells. Other *Fanc* genes, *Fanca*, *Fancd2*, *Fance* or *Fancf*, did not show any significant changes in mRNA expression in *Fancc*^{-/-} LTS-CD34⁺ and *Fancc*^{-/-} LTS-CD34⁻ cells as compared to *Fancc*^{+/+} cells. These results suggest that absence of *Fancc* influences mRNA expression levels of other *Fanc* genes in progenitors and total BM cells.

The breast cancer susceptibility genes, *Brca1* and *Brca2/Fancd1* are upregulated in *Fancc*^{-/-} hematopoietic cells**

We evaluated mRNA expression levels of the newly identified FA gene *Brca2/Fancd1*, and the FA-associated gene, *Brca1*, in *Fancc*^{+/+} and *Fancc*^{-/-} hematopoietic cells. We found that both *Brca2/Fancd1* and *Brca1* genes were expressed at low levels in all *Fancc*^{+/+} hematopoietic cell populations tested (ratios ranging from 0.29 to 0.36 and 0.09 to 0.24, respectively; Figure 4). We also evaluated mRNA levels in *Fancc*^{-/-} hematopoietic cells and found that both *Brca2/Fancd1* and *Brca1* are upregulated in *Fancc*^{-/-} total BM, progenitors and CD34⁺ cells. Of all the FA genes tested, *Fancd1/Brca2* and the FA associated gene *Brca1* seemed to be the most affected from absence of *Fancc* with 5 to 7 fold increase in gene expression levels in *Fancc*^{-/-} differentiated BM cells and progenitors (Lin⁻Thy1.2⁻), respectively (Table 2).

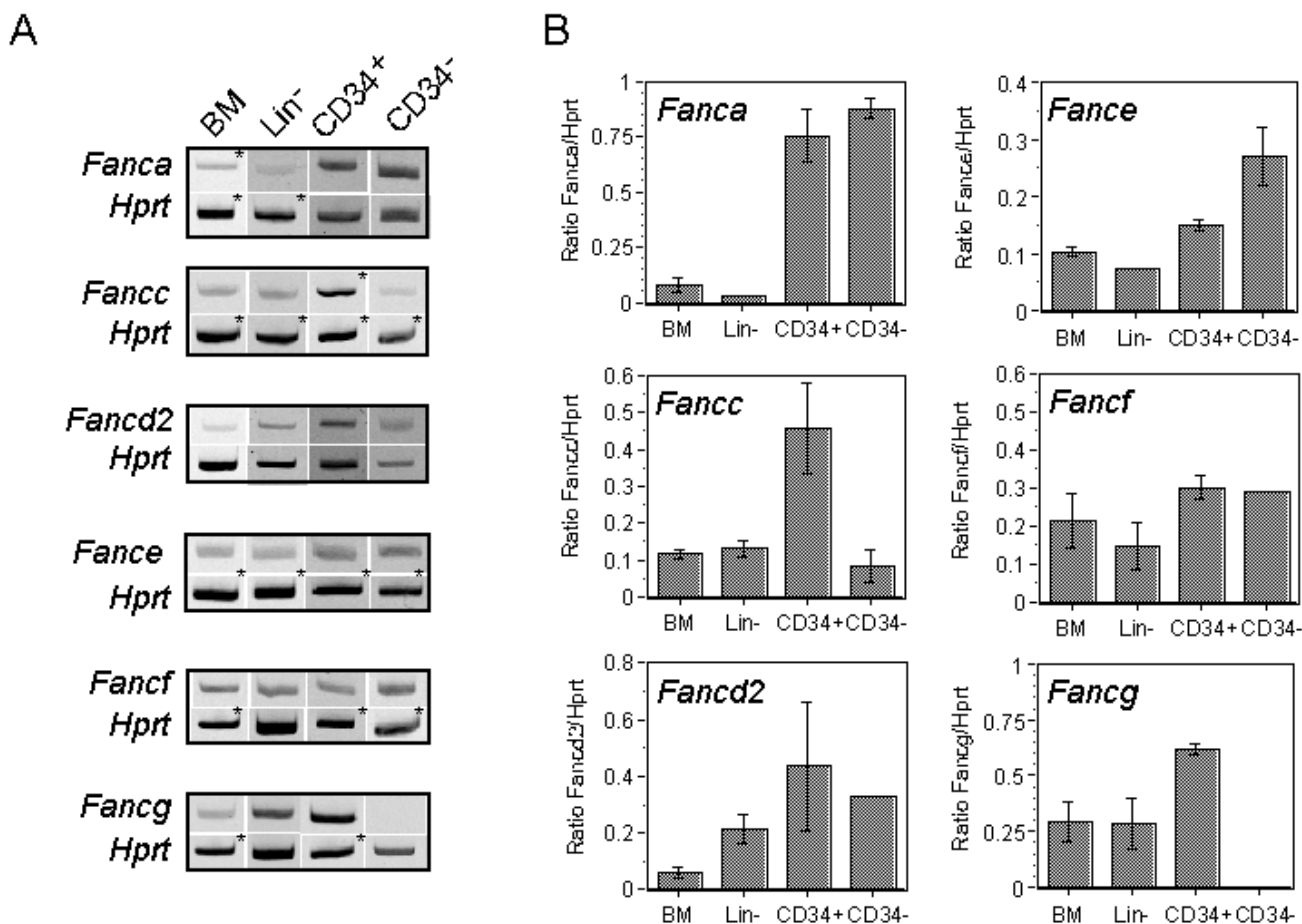


Figure 2
Fanc gene expression profiles in murine hematopoietic cells. (A) Representative gel electrophoresis of 10 ul (* represents 5 ul) of RT-PCR products from wild-type BM (BM), lineage and Thy1.2-depleted (Lin-) and sorted LTS-CD34+ (CD34+) and LTS-CD34- (CD34-) cells using specific Fanc and Hprt primers. (B) Mean relative expression of Fanc genes in wild-type hematopoietic cell populations from 2 to 10 separate determinations with the exception of *Fancf* in CD34- cells where values represent one determination. *Fancg* expression in CD34- cells was undetectable (n= 4).

It is difficult to know if the observed increase in *Brca* gene expression in *Fancc*^{-/-} hematopoietic cells is also reflected at the protein level. Since there are no commercially available antibodies directed against murine *Brca* proteins, we used anti-human BRCA1 and BRCA2 antibodies in Western blot and immunoprecipitation techniques and could not detect murine *Brca1* and *Brca2* proteins (data not shown). Thus, we could not evaluate protein expression levels in *Fancc*^{-/-} BM cells to determine if the observed mRNA expression profiles correlated with protein expression levels.

Nevertheless, these results suggest that absence of *Fancc* affects the regulation of other *Fanc* gene expression more

dramatically, *Brca2*/*Fancd1* and *Brca1*, through an unknown mechanism.

Discussion

Gene expression profiling and transcription regulation of different genes can be surveyed using several techniques such as Northern blots, differential display or microarray technology. Some may provide information on numerous genes simultaneously such as microarrays, but these tend to generate large and complex data. We chose a simple direct semiquantitative RT-PCR procedure to characterize specific gene expression levels in sorted hematopoietic stem and progenitor cell fractions. Since primitive hematopoietic stem cells are only obtainable in very limited amounts, PCR based methods are more sensitive and

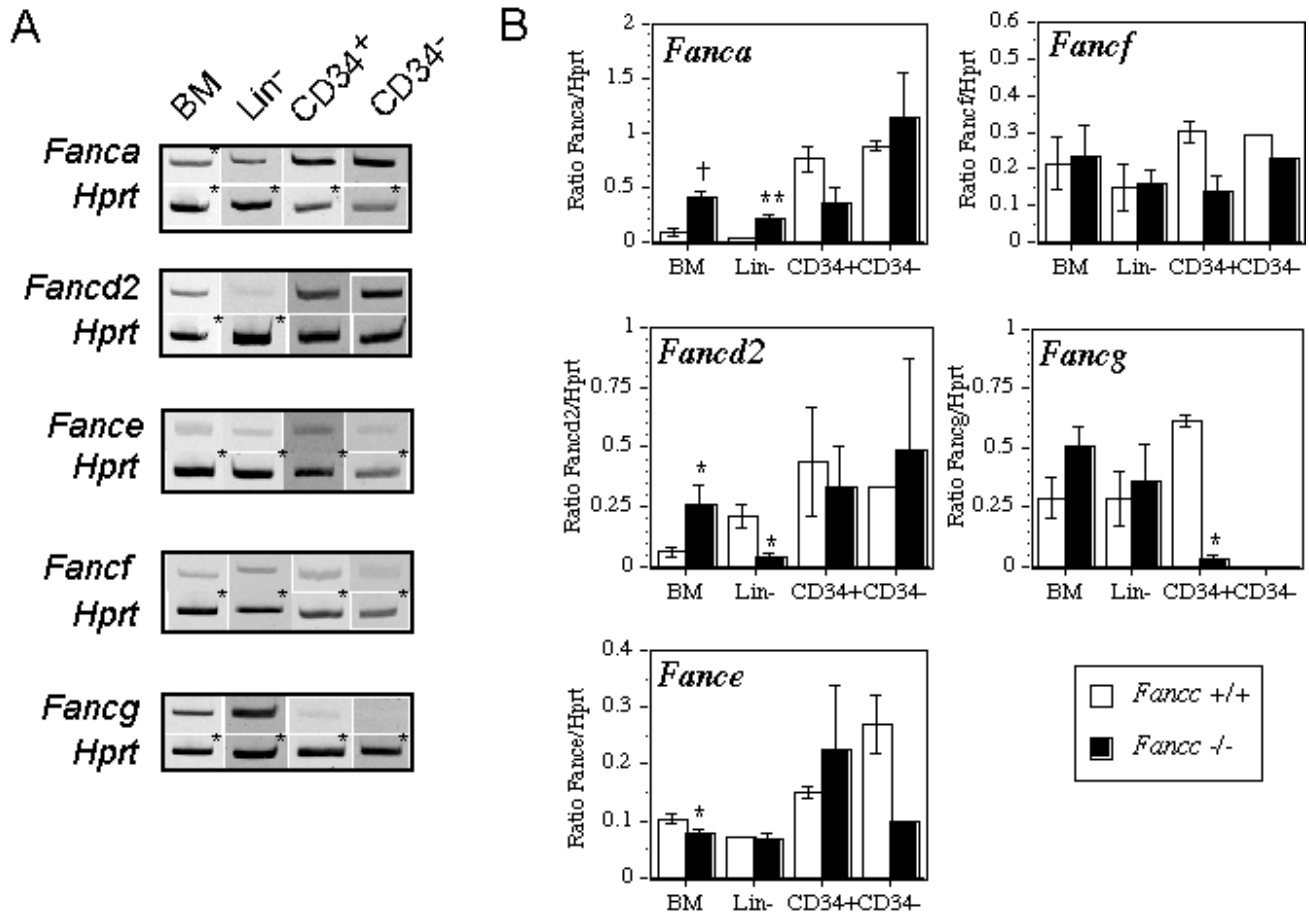
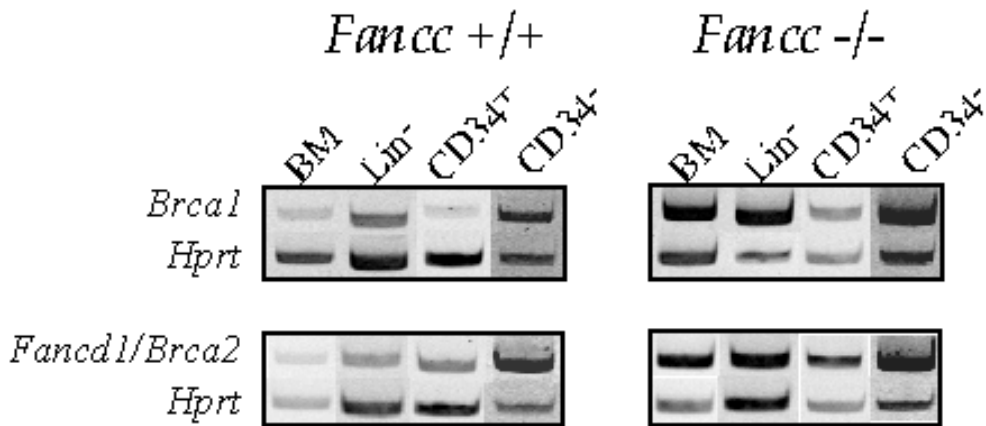


Figure 3
Comparative analysis of *Fanc* gene expression in hematopoietic cells from *Fancc*^{-/-} mice. (A) Representative gel electrophoresis of 10 ul (* represents 5 ul) of RT-PCR products from *Fancc*^{-/-} BM cells, BM; lineage and Thy1.2-depleted, Lin⁻; sorted LTS-CD34⁺, CD34⁺; and LTS-CD34⁻, CD34⁻ cells using specific *Fanc* and *Hprt* primers. (B) Quantitative comparison of the expression levels of *Fanc* genes in hematopoietic cell populations from *Fancc*^{+/+} and *Fancc*^{-/-} mice. Bars represent the mean value from 2 to 10 separate determinations with the exception of *Fance* and *Fancf* in CD34⁻ cells where values represent one determination. *Fancg* expression in CD34⁻ cells was undetectable in both *Fancc*^{+/+} (n= 4) and *Fancc*^{-/-} cells (n= 4). *Fancc*^{+/+} expression ratios are identical to those in Figure 2. Significant differences between *Fancc*^{-/-} and *Fancc*^{+/+} cells where * p < 0.05, ** p < 0.005 and † p < 0.001.

allow the detection of low abundance transcripts in small cell populations as compared to Northern blot analysis. In addition, we used single-round PCR amplifications followed by direct detection on polyacrylamid gels to keep relative abundance relationship between transcripts and to reduce variability between samples. The reverse transcription was performed using oligo (dT) primers and target gene expression ratios were based on the housekeeping gene, *Hprt*, transcription level (equivalent to 1.0) in the same sample, thus, allowing the comparison between different genes, between cell populations and between wild-type and *Fancc*^{-/-} cells.

In the current study, we tested the hypothesis that *Fanc* genes display a pattern of expression reminiscent of a role in hematopoietic stem cell differentiation and/or maintenance. Based on phenotypic analysis of *Fancc*^{-/-} mouse bone marrow, targeted deletion of *Fancc* alleles was shown to affect the number of primitive CD34⁺ cells suggestive of a role at the CD34⁺ stem cell differentiation stage [17]. We found that expression of most FA genes, notably, *Fanca*, *Fancc*, *Fancd2* and *Fancg*, are highly expressed in CD34⁺ stem cells as compared to more differentiated cell populations whereas *Fance* and *Fancf* are expressed at similar levels in all hematopoietic cell popu-

A



B

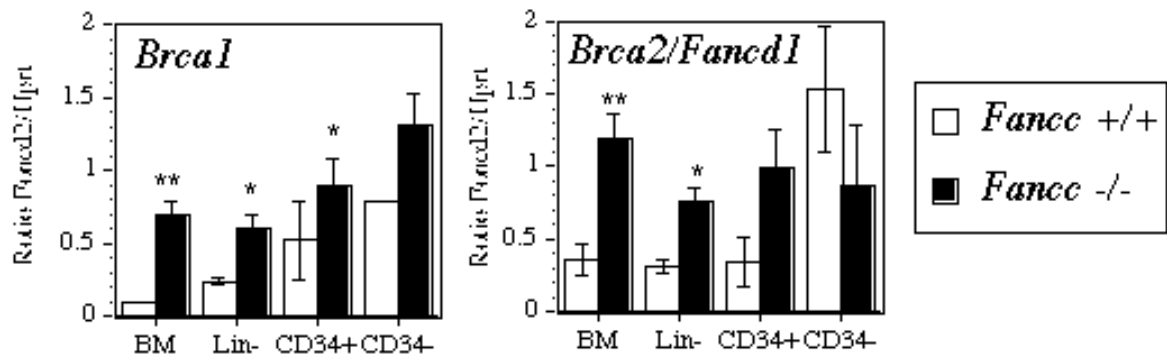


Figure 4

Comparative analysis of *Brca1* and *Brca2/Fancl1* gene expression in hematopoietic cells from *Fancc*^{-/-} mice. (A) Representative gel electrophoresis of 10 ul and 5 ul of RT-PCR products using specific *Brca1* or *Brca2/Fancl1* and *Hprt* primers, respectively, from *Fancc*^{-/-} BM cells, BM; lineage and Thy1.2-depleted, Lin⁻; sorted LTS-CD34⁺, CD34⁺; and LTS-CD34⁻, CD34⁻ cells. (B) Quantitative comparison of the expression levels of *Brca1* and *Brca2/Fancl1* genes in hematopoietic cell populations from *Fancc*^{+/+} and *Fancc*^{-/-} mice. Bars represent the mean value from 4 to 10 separate determinations. Absence of SEM bars represents values too low to appear. Significant differences between *Fancc*^{-/-} and *Fancc*^{+/+} cells where *p < 0.05 and **p < 0.005.

lations. We showed that *Fanca* was highly expressed in both long-term CD34⁻ and short-term CD34⁺ stem cells whereas *Fancc* and *Fanccg* appear to be downregulated in LTS-CD34⁻ stem cells and upregulated in CD34⁺ cells consistent with a role of *Fancc* at the CD34⁻ to CD34⁺ differentiation stage[17]. The fact that *Fanccg* is undetectable in CD34⁻ stem cells and highly expressed in CD34⁺ cells suggest that this gene may also function at the CD34⁺ stage. Taken together, these results suggest that *Fanc* genes are differently regulated in hematopoietic stem cells and suggest that each FA protein may act separately from the FA complex during hematopoiesis similarly to what has been

suggested for MMC sensitivity and cytokine signaling [26–29].

Our results showing increased *Fanca* and *Fancc* expression in primitive hematopoietic stem cells and reduced levels in differentiated BM cells are consistent with previous studies showing *Fanca* and *Fancc* expression in cells of mesenchymal origin that give rise to hematopoietic tissues during mouse development [30]. In addition, studies showing *Fancc* expression in areas undergoing osteogenesis, in regions containing dividing and/or differentiating cells, in progenitor/precursor cell popula-

tions and in primordial germ cell development are also consistent with our results showing elevated *Fancc* expression in primitive hematopoietic cells [20,32–34].

Mouse models for *Fanca*, *Fancc* and *Fanccg* have been generated [11–14] and were shown to display similar phenotypes, these include hypersensitivity to DNA crosslinking agents, increased spontaneous and induced chromosomal breakage and germ cell loss leading to reduced fertility. Studies using the *Fancc* $-/-$ mice have shown that lack of *Fancc* resulted in a reduced number of CD34⁺ stem cells [17]. Since the murine CD34 marker has been associated not only with the differentiation state or short-term repopulating cells, but also with activation and/or cycling state [24,35], the reduced number of CD34⁺ cells in *Fancc* $-/-$ mice and the fact that *Fancc* $-/-$ stem/progenitor cells (Lin^c-Kit⁺Sca-1⁺) were shown to be less quiescent [36] may also reflect an altered activation state. Murine *Brca1* and *Brca2* mRNA expression was shown to be up-regulated in rapidly proliferating cells indicative of a role in proliferation and differentiation control [37]. Also, murine *Fanccg* mRNA was shown to be expressed in highly proliferative and embryonic tissues [31]. Since *Fancc* $-/-$ progenitor/stem cells were shown to be less quiescent [36], our results showing increased *Brca1* and *Brca2/Fancc1* mRNA expression levels in *Fancc* $-/-$ BM cells would be consistent with an altered proliferation state. However, this is not the case with *Fanccg* expression pattern, where mRNA levels were found to be dramatically reduced in *Fancc* $-/-$ LTS-CD34⁺ cells. Thus, the increased expression levels of *Brca1* and *Brca2/Fancc1* in *Fancc* $-/-$ cells and reduced *Fanccg* expression in CD34⁺ cells may not be a consequence of an increase in proliferation but may reflect dysregulated gene expression due to absence of *Fancc*. The role that *Fancc* might have on other FA gene expression has yet to be determined.

Conclusions

Together our results show that FA genes are regulated at the mRNA level that increased *Fancc* expression in LTS-CD34⁺ cells correlates with a role at the CD34⁺ differentiation stage and that lack of *Fancc* downregulates *Fanccg* expression in CD34⁺ stem cells but increases *Brca1* and *Brca2/Fancc1* expression in all BM cell populations. Differential expression of FA genes in hematopoietic cell population suggest that FA genes may have distinct function at different stem cell stages but that *Fancc* and *Fanccg* may function specifically at the CD34⁺ differentiation stage.

Competing interests

None declared.

Authors' contributions

MA performed hematopoietic cell purifications, mRNA extractions, RT-PCR and analysis of *FA* genes. ML performed hematopoietic cell purifications, mRNA extraction, RT-PCR and analysis of *Brca* genes. IB performed Western blot and immunoprecipitation studies. MCD was responsible for maintaining the mouse colony and participated in hematopoietic cell purifications. MC conceived the study and participated in its design and coordination. All authors read and approved the final manuscript.

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