Molecular basis of crosstalk between oncogenic Ras and the master regulator of hematopoiesis GATA-2

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Abstract

Disease mutations provide unique opportunities to decipher protein and cell function. Mutations in the master regulator of hematopoiesis GATA-2 underlie an immunodeficiency associated with myelodysplastic syndrome and leukemia. We discovered that a GATA-2 disease mutant (T354M) defective in chromatin binding was hyperphosphorylated by p38 mitogen-activated protein kinase. p38 also induced multisite phosphorylation of wild-type GATA-2, which required a single phosphorylated residue (S192). Phosphorylation of GATA-2, but not T354M, stimulated target gene expression. While crosstalk between oncogenic Ras and GATA-2 has been implicated as an important axis in cancer biology, its mechanistic underpinnings are unclear. Oncogenic Ras enhanced S192-dependent GATA-2 phosphorylation, nuclear foci localization, and transcriptional activation. These studies define a mechanism that controls a key regulator of hematopoiesis and a dual mode of impairing GATA-2-dependent genetic networks: mutational disruption of chromatin occupancy yielding insufficient GATA-2, and oncogenic Ras-mediated amplification of GATA-2 activity.

Keywords GATA factor; GATA-2; p38; Ras; transcription

Subject Categories Cancer; Post-translational Modifications, Proteolysis & Proteomics; Transcription

DOI 10.15252/embr.201438808 | Received 21 March 2014 | Revised 19 June 2014 | Accepted 23 June 2014 | Published online 23 July 2014

EMBO Reports (2014) 15: 938–947

See also: C Baumgartner & M Baccarini (September 2014)

Introduction

The establishment and maintenance of genetic networks must be exquisitely orchestrated to ensure the fidelity of biological processes. While intense efforts are documenting genetic networks, many questions remain unanswered regarding how master regulators establish/maintain networks, how signaling mechanisms

control networks, and how alterations influence vulnerable nodes within a network. As GATA transcription factors control genetic networks in diverse contexts [1–3], and pathological GATA factor mutants have been described [4, 5], it is instructive to consider the consequences of such mutations.

Of the six mammalian GATA factors, GATA-1, GATA-2, and GATA-3 have important roles in regulating hematopoiesis [1]. GATA-1 regulates erythropoiesis, megakaryopoiesis, and the development of eosinophils and mast cells, while GATA-2 controls primitive and definitive hematopoiesis [6]. GATA-2 induces HSC generation in the AGM and HSPCs in the fetal liver [7–10]. As *Gata2*^{+/-} HSCs are impaired [11, 12], and GATA-2 overexpression suppresses hematopoiesis [13, 14], increases and decreases in GATA-2 disrupt hematopoiesis.

Alterations in GATA-2 expression are implicated in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [4]. Elevated GATA-2 expression correlates with AML severity [15, 16]. Heterozygous GATA-2 mutations cause an immunodeficiency (MonoMAC or Emberger syndrome) associated with MDS/AML [17–20]. While these mutations, for example, T354M, often occur within the GATA-2 DNA binding zinc finger, mutations of the +9.5 *cis*-element can also cause MonoMAC [8, 21].

GATA-2(T354M) expressed in 293 cells was not competent for DNA binding [17]. However, certain factors defective in DNA binding can function *in vivo* [22, 23], and chromatin occupancy can be regulated independent of DNA binding [24, 25]. While dissecting how leukemogenic mutations alter GATA-2, we discovered that the functionally impaired T354M mutant was hyperphosphorylated, and a Ras-p38-dependent phosphorylation mechanism amplified wild-type GATA-2 activity.

Results and Discussion

GATA-2 disease mutation reduces chromatin occupancy and target gene regulation

To determine how the T354M mutation (Fig 1A) influences GATA-2 function, we expressed GATA-2(T354M) in G1E proerythroblasts,

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Figure 1. T354M mutation attenuates GATA-2 chromatin occupancy and endogenous target gene activation.

A T354M mutant.

B Western blot analysis with anti-GATA-2 or anti-HA antibodies of HA-GATA-2 and HA-GATA-2(T354M) transiently expressed in G1E cells.

C Quantitative ChIP analysis of HA-GATA-2 and HA-GATA-2(T354M) occupancy in G1E cells transiently expressing HA-GATA-2 or HA-GATA-2(T354M) ($n = 4$, mean $\pm$ SE).

D Western blot analysis of HA-GATA-2 and HA-GATA-2(T354M) transiently expressed in MAE cells.

E Quantitation of GATA-2 target gene expression. Real-time RT-PCR analysis in MAE cells expressing HA-GATA-2 or HA-GATA-2(T354M) ($n = 9$, mean $\pm$ SE) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, relative to the empty vector.

F ChIP-seq analysis of GATA-2 occupancy at *HDC* and *IL13* loci in human CD34-positive hematopoietic cells [34] and HUVECs [33].

Data information: Significance of the differences was estimated using Paired Student's t -test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

which express endogenous GATA-2 [26]. Western blot analysis with anti-GATA-2 (Fig 1B, left) and anti-HA (Fig 1B, right) antibodies revealed GATA-2(T354M) migration as two bands—with a mobility indistinguishable from wild-type GATA-2 or with a slower mobility. Wild-type GATA-2 also migrated as two bands,

although the upper band was less abundant versus GATA-2 (T354M).

Using the anti-HA antibody and a quantitative ChIP assay, we compared the capacities of expressed GATA-2 or GATA-2(T354M) to occupy chromatin [27–29] (Fig 1C). Whereas GATA-2 occupied

Figure 2. p38-mediated GATA-2 and GATA-2(T354M) hyperphosphorylation and target gene regulation.

- A Total protein from 293 cells expressing HA-GATA-2, HA-GATA-2(T354M), or empty vector was incubated with or without λ-phosphatase and analyzed by Western blotting with anti-HA antibody.
- B Left, Western blot analysis of wild-type GATA-2 and HA-GATA-2(T354M) from 293 cells treated with vehicle or p38 inhibitor SB203580. Right, densitometric analysis. The ratio of intensities of the T354M upper to lower bands from control untreated cells was designated as 1 ($n = 3$, mean $\pm$ SE).
- C Total protein from G1E cells expressing HA-GATA-2, HA-GATA-2(T354M), or empty vector was incubated with or without λ-phosphatase. Proteins were resolved by SDS-PAGE and analyzed by Western blotting with anti-HA antibody.
- D Total protein from G1E cells expressing HA-GATA-2(T354M) was incubated with or without λ-phosphatase and analyzed by Western blotting with anti-GATA-2 antibody.
- E Left, Western blot analysis of wild-type GATA-2 and HA-GATA-2(T354M) from G1E cells treated with vehicle or p38 inhibitor SB203580. Right, densitometric analysis. The ratio of intensities of the T354M upper to lower bands from control untreated cells was designated as 1 ($n = 3$, mean $\pm$ SE).
- F Western blot analysis of GATA-2 in G1E cells treated with SB203580.
- G Western blot analysis of HA-GATA-2(T354M) and p38 in G1E cells infected with sh-luc virus or sh-p38α virus.
- H Real-time RT-PCR analysis of p38 mRNA and transcripts of GATA-2 target genes in G1E cells infected with sh-luc virus or sh-p38α virus ($n = 3$, mean $\pm$ SE).
- I Left, Western blot analysis of HA-GATA-2 in G1E cells treated with okadaic acid. Right, densitometric analysis. The upper to lower band ratio from control was designated as 1 ($n = 4$, mean $\pm$ SE). Cells were treated with okadaic acid for 24 h. The viability was more than 75%.
- J Left, Western blot analysis of endogenous GATA-2 in G1E cells treated with okadaic acid (8% gel was used instead of 10% used in D and F). Right: Real-time RT-PCR analysis in okadaic acid-treated G1E cells ($n = 4$, mean $\pm$ SE).

Data information: Significance of the differences was estimated using Paired Student's *t*-test. * $P < 0.05$.

the -3.9 , -2.8 , -1.8 , and $+9.5$ GATA switch sites of the *Gata2* locus and the *Lyl1* locus, little to no GATA-2(T354M) chromatin occupancy was detected (Fig 1C). GATA-2(T354M) was expressed at least as high as GATA-2 (Fig 1B, right).

The reduced GATA-2(T354M) chromatin occupancy suggested a defect in target gene regulation. A system does not exist to test whether exogenously expressed GATA-2 can regulate endogenous target genes. G1E cells express high-level endogenous GATA-2 and are not ideal for addressing this issue. We developed a mouse aortic endothelial (MAE) cell system in which exogenous GATA-2 regulates endogenous target genes. Immortalized MAE cells bear a normal endothelial phenotype [30] and express endogenous GATA-2 considerably lower than G1E cells.

Expression profiling in PECAM1⁺ cells and Aorta Gonad Mesonephros (AGM) from control and $+9.5^{-/-}$ embryos identified endogenous GATA-2 target genes [7, 8]. We tested whether GATA-2 regulates these genes in MAE cells. Transiently expressed GATA-2 and GATA-2(T354M) in MAE cells migrated as two bands, analogous to G1E cells, with the upper band more abundant with GATA-2 (T354M) versus GATA-2 (Fig 1D). GATA-2 activated *Hdc*, *IL13*, *Gfi1b*, and *Gfi1* expression 200, 10, 11, and 10-fold, respectively, whereas GATA-2(T354M) had little activity (Fig 1E). The GATA-2 target genes *Gfi1b* and *Gfi1* [7, 27, 31] function in hemogenic endothelium to control HSC generation [32]. Although *Hdc* and *IL13* are not established GATA-2 targets, ChIP-seq analysis in HUVECs [33] and human CD34⁺ hematopoietic precursors [34] revealed GATA-2 occupancy at *Hdc* and *IL13* loci (Fig 1F and Supplementary Fig S1A). Cotransfection of GATA-2(T354M) did not influence GATA-2-mediated *Hdc* induction (Supplementary Fig S1B). Thus, T354 is critical for GATA-2 chromatin occupancy and target gene regulation.

p38 mitogen-activated protein kinase-dependent GATA-2 multi-site phosphorylation governs GATA-2 activity

To determine the basis of the T354M-enhanced mobility shift, GATA-2 or GATA-2(T354M) was expressed in 293 cells and cell lysates were treated with λ -phosphatase. λ -phosphatase abolished the GATA-2(T354M) upper band and increased GATA-2 mobility equivalent to λ -phosphatase-treated GATA-2(T354M) (Fig 2A). Screening signaling pathway inhibitors revealed that the p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580, but not ERK (U0126) or JNK (SP600125) inhibitors, decreased the upper and

increased the lower band (Fig 2B, Supplementary Fig S2A and B). In G1E lysates, λ -phosphatase (Fig 2C) and SB203580 (Fig 2E) decreased abundance of the GATA-2(T354M) upper band and increased endogenous GATA-2 mobility (Fig 2D and F). The MDS mutant GATA-2(Δ 355T) [17] and a C349A DNA binding-defective mutant were hyperphosphorylated (Supplementary Fig 2C). p38 α knockdown reduced GATA-2(T354M) hyperphosphorylation (Fig 2G) and wild-type GATA-2 regulation of target genes (Fig 2H). The protein phosphatase inhibitor okadaic acid induced GATA-2 hyperphosphorylation and GATA-2 target gene expression (*Hdc* and *Gfi1*) (Fig 2I and J). Thus, GATA-2 is phosphorylated in a p38 α -dependent manner, and leukemogenic mutations that abrogate chromatin occupancy promote p38 α -dependent phosphorylation and/or impair GATA-2 dephosphorylation (Fig 2). These results suggest that p38 α phosphorylation of GATA-2 controls wild-type GATA-2 activity.

Mass spectrometry was used to identify GATA-2 and GATA-2 (T354M) phosphorylated residues (Fig 3A). Analysis of proteins immunopurified from 293 cells revealed multi-site phosphorylation (Fig 3B) that was more abundant in GATA-2(T354M) versus GATA-2 (Fig 3C). Unbiased deletion mutants and point mutants that eliminate potential phosphorylatable residues were analyzed to delineate requirements for GATA-2(T354M) hyperphosphorylation (Supplementary Table S1). S192A was the only point mutation that abrogated the shift (Fig 3D). While deleting amino acids 61–120 abrogated the shift, a smaller deletion including S73 and S119 did not (Supplementary Fig S3B–D and Supplementary Table S1). S192A mutation also increased GATA-2 mobility, resembling λ -phosphatase treatment (Fig 3D and E). Phosphonet (<http://www.phosphonet.ca/>) analysis suggested that S192 can be phosphorylated by p38 α . Though GATA-2 S192 is evolutionarily conserved, other GATA factors lack a comparable serine. GATA-3 contains a potentially analogous threonine (Fig 3F). S192A reduced GATA-2 phosphorylation at S73, S119, and S290 (Fig 3G and H). Thus, Ser192 is phosphorylated, promotes multi-site phosphorylation, and is required for GATA-2(T354M) hyperphosphorylation.

In MAE cells, GATA-2(S192A) (Fig 3I) had some capacity to activate *Hdc* and *IL13*, but its activity was significantly less than GATA-2 (Fig 3J). In G1E cells, HA-GATA-2(S192A) chromatin occupancy was ~40% less than HA-GATA-2 (Fig 3K and L). As Ser192 is required for GATA-2(T354M) hyperphosphorylation, and GATA-2 (T354M) is defective in chromatin binding and transcriptional

Figure 3. Ser192-mediated GATA-2 phosphorylation.

- A Immunoprecipitation of HA-GATA-2 and HA-GATA-2(T354M) expressed in 293 cells. Proteins were detected by Western blotting with anti-HA antibody.
- B Phosphorylated residues detected by LC-ESI MS/MS.
- C Relative phosphorylation of S73, S119, S192, and S290 in GATA-2 and T354M ($n = 3$, mean $\pm$ SE).
- D Western blot analysis with anti-HA antibody of proteins expressed in G1E cells.
- E 293 cell proteins incubated with or without λ -phosphatase and analyzed by Western blotting with anti-HA antibody.
- F Murine GATA factor amino acid sequences (top) and GATA-2 sequences from multiple species (bottom).
- G Immunoprecipitation of HA-GATA-2 and HA-GATA-2(S192A) expressed in 293 cells. Proteins were detected by Western blotting with anti-HA antibody.
- H Relative phosphorylation of S73, S119, S192, and S290 in GATA-2 and S192 mutant determined by mass spectrometry analysis ($n = 3$, mean $\pm$ SE).
- I Western blot analysis with anti-HA antibody of wild-type and mutant proteins expressed in MAE cells.
- J Real-time RT-PCR analysis of *Hdc* and *IL13* mRNA levels in MAE cells expressing wild-type or mutant proteins ($n = 3$, mean $\pm$ SE).
- K Quantitative ChIP analysis of HA-GATA-2 and HA-GATA-2(S192A) occupancy with anti-HA antibody in G1E cells expressing HA-GATA-2 or HA-GATA-2(S192A) ($n = 5$, mean $\pm$ SE). The Western blot (anti-HA antibody) in the inset illustrates GATA-2 and S192A expression in representative samples used for ChIP.
- L S192-mediated multi-site GATA-2 hyperphosphorylation.

Data information: Significance of the differences was estimated using Paired Student's t-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 4. S192 requirement for oncogenic Ras-induced GATA-2 activity.

- A** Total protein from 293 cells expressing HA-GATA-2, with or without H-Ras(G12V), was incubated with or without λ-phosphatase and analyzed by Western blotting with anti-HA antibody.
- B** Western blot analysis with anti-HA antibody of proteins expressed in G1E cells with or without H-Ras(G12V).
- C** Left, Western blot analysis of G1E cells expressing HA-GATA-2 and H-Ras(G12V) with or without SB203580. Right, densitometric analysis. The ratio of intensities of the upper to lower bands from Ras(G12V)-expressing cells was designated as 1 ($n = 3$, mean $\pm$ SE).
- D** Influence of varying HA-GATA-2 expression vector concentration with or without H-Ras(G12V) on *Hdc* induction in MAE cells ($n = 3$, mean $\pm$ SE). Left, real-time RT-PCR analysis of *Hdc* mRNA level. Right, Western blot analysis with anti-HA antibody.
- E** Top, Western blot analysis of HA-GATA-2 and HA-GATA-2(T354M) proteins expressed in MAE cells with or without H-Ras(G12V). Bottom, real-time RT-PCR analysis of *Hdc* mRNA and *Gfi1* mRNA levels in MAE cells expressing HA-GATA-2 and HA-GATA-2(T354M), with or without H-Ras(G12V) ($n = 4$, mean $\pm$ SE). * $P < 0.05$. Inset, expanded view of low-level *Hdc* expression.
- F** Top, Western blot analysis of HA-GATA-2 and HA-GATA-2(S192A) proteins expressed in MAE cells with or without H-Ras(G12V). Bottom, real-time RT-PCR analysis of *Hdc* mRNA levels in MAE cells expressing HA-GATA-2 and HA-GATA-2(S192A), with or without H-Ras(G12V) ($n = 3$, mean $\pm$ SE).
- G** Real-time RT-PCR analysis of *Hdc*, *IL13*, and *Gfi1* mRNA levels in G1E cells expressing HA-GATA-2, with or without H-Ras(G12V) ($n = 4$, mean $\pm$ SE).

Data information: Significance of the differences was estimated using Paired Student's *t*-test. * $P < 0.05$, ** $P < 0.01$.

activation, chromatin binding and transcriptional activation are not required for hyperphosphorylation. A S192A/T354M mutant was also inactive (Supplementary Fig S3E).

GATA-2 S192 integrates oncogenic signals

GATA-2-Ras interactions are implicated in non-small cell lung [35] and colon cancer [36]. Because Ras signaling activates p38 α , p38 α -dependent Ser192 phosphorylation may underlie Ras-GATA-2 crosstalk. Constitutively active Ras [Ras(G12V)] induced the upper GATA-2 band, which was abrogated by λ -phosphatase (Fig 4A), and was indistinguishable from the T354M-induced band; both required Ser192 and amino acids 61–120 (Figs 3D and 4B and Supplementary Fig S3C) and were reduced by SB203580 (Figs 2B and 4C). Though the function of amino acids 61–120 is not established, GATA factor N-termini can facilitate transactivation [4]. Ras (G12V) expression in MAE cells enhanced GATA-2-mediated *Hdc* and *Gfi1* expression 10 and 5-fold, respectively, without affecting the weak GATA-2(T354M) activity (Fig 4D and E). S192A attenuated GATA-2/Ras(G12V)-mediated *Hdc* induction (Fig 4F). Ras (G12V) expression in G1E cells enhanced GATA-2-mediated *Hdc*, *IL13*, and *Gfi1* expression 6, 3, and 2.5-fold, respectively (Fig 4G).

Ras(G12V) increased GATA-2, but not S192A and Δ 61–120, localization into nuclear foci [13.9–25.4%, – and + Ras(G12V), respectively] (Fig 5A and B). SB203580 reduced GATA-2 foci localization (Fig 5C and D). The foci partially colocalized with serine 2-phosphorylated Pol II, an active transcription marker (Fig 5E). Thus, p38 α and Ras(G12V) regulate GATA-2 phosphorylation, subnuclear localization, and transcriptional activation (Fig 5F).

Mechanistic considerations

Dissecting the deficits of a GATA-2 disease mutant uncovered a signal-dependent GATA factor pathway. While GATA-2 can be phosphorylated by cyclin-dependent kinases [37], Akt [38] and MAPKs [39], the modified residues and mechanistic consequences were not known. p38-dependent GATA-3 phosphorylation facilitates importin- α binding and cytoplasmic to nuclear translocation [40] and regulates LT-HSC self-renewal [41].

As p38 α inhibition attenuated GATA-2 hyperphosphorylation and target gene activation (Figs 2 and 5F), this mechanism controls wild-type GATA-2 function. Besides regulating LT-HSC self-renewal via targeting GATA-3 [41], p38 mediates reactive oxygen species-dependent reductions in HSC lifespan [42] and function of the HSC regulator thrombopoietin [43]. Inhibiting p38 restores hematopoiesis in defective MDS progenitors [44]. Elevated p38 activity may therefore target Ser192, increasing GATA-2 activity and deregulating the genetic network. By decreasing S192-dependent phosphorylation, p38 inhibition would reduce GATA-2 activity to a level compatible with normal hematopoiesis.

Ser192-dependent phosphorylation facilitates GATA-2 chromatin occupancy and target gene activation. Ser192 mediates GATA-2 and T354M phosphorylation. As Ser192 increased GATA-2 activity, Ser192-dependent hyperphosphorylated isoform is a minority of GATA-2, and phosphatase inhibition induced GATA-2 hyperphosphorylation, efficient GATA-2 dephosphorylation or dephosphorylation of a component in the activation mechanism restricts GATA-2 activity. This mechanism would limit GATA-2 activity and HSPC

Figure 5. Ras-p38-regulated GATA-2 subnuclear localization.

- A Immunofluorescence analysis with anti-HA antibody in G1E cells expressing HA-GATA-2 and mutant proteins with or without H-Ras(G12V). Scale bars: 5 μ m.
- B The percentage of cells exhibiting foci, based on analysis of > 150 cells ($n = 3$, mean $\pm$ SE).
- C Immunofluorescence analysis with anti-HA antibody in G1E cells expressing HA-GATA-2 and H-Ras(G12V) with or without SB203580. Scale bars: 5 μ m.
- D The percentage of cells exhibiting foci, based on examining > 150 cells ($n = 3$, mean $\pm$ SE).
- E Co-immunostaining with anti-HA and anti-serine 2-phosphorylated Pol II antibody in G1E cells expressing HA-GATA-2 and H-Ras(G12V). Arrows, colocalization signals. Bars: 5 μ m.
- F Ras-p38 axis enhances GATA-2 phosphorylation, subnuclear localization, and target gene activation.

Data information: Significance of the differences was estimated using Paired Student's *t*-test. $P < 0.05$.

generation/function. Since hyperphosphorylated T354M is defective in chromatin binding/target gene regulation, failure to dephosphorylate T354M would be inconsequential.

GATA-2–cancer links [4, 15–20, 45, 46], and oncogenic Ras targeting of Ser192, suggest the Ras-p38-GATA-2 axis may have vital roles in disease etiology. It will be instructive to decipher how

GATA-2 phosphorylation is opposed physiologically and how this balance is skewed in GATA-2-dependent pathologies not involving GATA-2 mutations that impair chromatin occupancy.

Materials and Methods

Experimental procedures are described in detail in the Supplementary Materials and Methods.

Cell culture

G1E and MAE cells were maintained as described [8, 29].

Plasmids [8]

GATA-2 cDNA was cloned into pcDNA4TOFHA vector (from Dr. Danny Reinberg, NYU). H-Ras and H-Ras(G12V) expression vectors were from Dr. Jing Zhang (UW-Madison).

Quantitative chromatin immunoprecipitation (ChIP)

ChIP analysis in G1E cells was conducted as described [47].

Protein analysis

For analysis of phosphorylation, protein was prepared with RIPA buffer containing 50 mM β -glycerophosphate, 50 mM sodium fluoride, and 200 μ M sodium vanadate. For phosphatase treatment, protein was incubated with 800 units of λ -phosphatase (New England Biolabs) in 30 μ l (293 cells) and 80 μ l (G1E) at 30°C for 90 min.

Immunoprecipitation

Protein prepared by lysing 293 cells in RIPA buffer was immunoprecipitated with preimmune serum or rabbit anti-GATA-2 antibody [29].

Statistical analysis

Statistical significance was determined by Paired Student's *t*-test using GraphPad software (www.graphpad.com).

ChIP-Seq

ChIP-seq profiles for GATA-2 in HUVEC cells [33] and human CD34-positive cells [34] were generated using the UCSC Genome Browser (<http://genome.ucsc.edu/>) (GEO accession GSE29531).

Mass spectrometry

GATA-2 was excised and analyzed by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI MS/MS). Samples (3 μ l) were resolved on analytical columns, eluted peptides were introduced into the mass spectrometer via electrospray ionization, and peptide ions were subjected to tandem mass spectrometry. Raw MS/MS spectra were processed with Proteome Discoverer (v.1.4.0.288, Thermo Fisher Scientific). Peak lists were searched

against a database containing wild-type and mutant mouse GATA-2 sequences. Peptides were filtered by XCorr scores, and positions of phosphorylated residues were determined by site probabilities calculated by phosphoRS [48] in Proteome Discoverer. Peak areas were calculated by 'Precursor Ions Area Detector' node in Proteome Discoverer and normalized to the average protein amount in each MS analysis [49].

Supplementary information for this article is available online: <http://embor.embopress.org>

Acknowledgements

This work was supported by grants NIH DK68634, DK50107, and from the Midwest Athletes Against Childhood Cancer Organization to EHB and S10 RR029531 to LL. We are grateful to the UW Pharmacy Analytical Center for access to the Q Exactive Orbitrap mass spectrometer.

Author contributions

KRK devised experiments, conducted experiments, analyzed data, and wrote the paper. MEB participated in data analysis. CY devised experiments, conducted experiments and analyzed data. LL devised experiments and analyzed data. EHB devised experiments, analyzed data, and wrote the paper.

Conflict of interest

The authors declare that they have no conflict of interest.

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