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Heme controls the regulation of protein tyrosine kinases Jak2 and Src

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ABSTRACT

Protein tyrosine kinases play key roles in many molecular and cellular processes in diverse living organisms. Their proper functioning is crucial for the normal growth, development, and health in humans, whereas their dysfunction can cause serious diseases, including various cancers. As such, intense studies have been performed to understand the molecular mechanisms by which the activities of protein tyrosine kinases are regulated in mammalian cells. Particularly, small molecules that can modulate the activity of tyrosine kinases are of great importance for discovering therapeutic drug candidates for numerous diseases. Notably, heme cannot only serve as a prosthetic group for hemoglobins and enzymes, but it also is a small signaling molecule that can control the activity of diverse signaling and regulatory proteins. Using a computational search, we found that a group of non-membrane spanning tyrosine kinases contains one or more CP motifs that can potentially bind to heme and mediate heme regulation. We then used experimental approaches to determine whether heme can affect the activity of any of these tyrosine kinases. We found that heme indeed affects the phosphorylation of key tyrosine residues in Jak2 and Src, and is therefore able to modulate Jak2 and Src activity. Further experiments showed that Jak2 and Src bind to heme and that the presence of heme alters the sensitivity of Jak2 and Src to trypsin digestion. These results suggest that heme actively interacts with Jak2 and Src and alters their conformation.

1. Introduction

Tyrosine kinases play crucial roles in signal transduction in virtually all mammalian cells. They coordinate cellular responses to a wide array of extracellular stimuli, and regulate cell growth, survival, differentiation, migration, and metabolism [1–7]. Deregulated expression or activity of tyrosine kinases can promote serious diseases, particularly cancers [8–18]. As such, understanding the molecular mechanisms by which the activity of tyrosine kinases is controlled is a key subject in cancer biology. Heme, iron protoporphyrin IX, is well known to be an essential molecule for many living organisms, particularly mammals [19]. It serves as a prosthetic group in various types of proteins and enzymes, including hemoglobin, myoglobin, cytochromes, catalase, and peroxidase [19]. Remarkably, recent evidence shows that heme controls the expression and activity of an increasing number of key regulators of diverse cellular processes [20,21]. For example, heme binds to and directly regulates the activities of several transcriptional regulators, including the yeast transcriptional regulator Hap1, the mammalian transcriptional repressor Bach1, and the mammalian nuclear receptor Rev-erbα [22–24]. Besides transcriptional regulators, heme also directly controls the activities of many other kinds of regulators, including the heme-regulated eIF2α kinase (HRI) and the essential miRNA processing factor DGCR8 [25–28]. Heme is required for the activation/phosphorylation of Raf, MEK1/2, and ERK1/2 – components of the mitogen-activated protein (MAP) kinase signaling pathway, in both the human epithelial cervix carcinoma HeLa cells and in the nerve growth factor-induced rat pheochromocytoma PC12 cells [29,30]. Clearly, heme has the potential to affect, directly or indirectly, the activities of many key cellular signaling proteins and regulators. It would be important to determine if heme also controls the activity of other key cellular regulators, such as tyrosine kinases that are crucial to cellular functions and to numerous disease processes.

However, experimental methods to systematically identify heme-regulated proteins are limited by technical difficulties to identify heme-regulated proteins by conventional methods. Thus, alternative methods may be useful for identifying heme-regulated proteins. To this end, we used computational methods to identify proteins that contain the CP motif – a motif shared by many known proteins that are regulated by heme, including Hap1, Bach1, and HRI [20]. In the case of Hap1 and Bach1, it has been demonstrated that one or more of the CP motifs bind to heme and mediate heme regulation of their activity [24,31,32]. This motif allows proteins to bind to heme reversibly and transiently and to respond to changes of cellular heme concentration dynamically, which should be a unique property of heme-regulated proteins [23]. Therefore, we

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reasoned that we may use the presence of CP motifs in proteins to narrow down candidates for potential heme-regulated proteins. We therefore did a computational search of all known human proteins to identify proteins that contain one or more CP motifs. We found that 23 non-membrane spanning protein tyrosine kinases contain one or more CP motifs. We then examined experimentally whether some of the important tyrosine kinases are actually regulated by heme. Our results demonstrated that heme is critical for the regulation of both Jak2 and Src kinase activities.

2. Materials and methods

2.1. Cell culture, antibodies, and proteins

HeLa cells (human epithelial cervix carcinoma) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% sodium pyruvate, 1% L-glutamine, 1% HEPES, and 100 U penicillin/100 μg streptomycin per ml. Heme-depleted serum was prepared as described previously [30]. Briefly, serum was treated with ascorbic acid for 3 h, followed by dialysis against PBS and filter sterilization. Heme depletion was monitored by measuring optical absorbance at 405 nm. The absorbance was reduced at least 50% after treatment with ascorbic acid.

All antibodies for Western blotting were purchased from Cell Signaling Technology. Purified full-length Src and Jak2 fragment containing residues 808-end were purchased from Millipore and Enzo Life Sciences, respectively. Carbonic anhydrase, BSA, and β-amylase were purchased from Sigma. Sequencing grade trypsin was purchased from Promega. Hemin agarose beads were purchased from Sigma.

2.2. RNAi knockdown of heme synthesis in HeLa cells

Target sequences (five for each gene) for knocking down 5-aminolevulinic acid synthase (ALAS1, non-erythroid form) and porphobilinogen deaminase (PBGD) were selected by using the computation program provided by Sigma–Aldrich. Then shRNA pLKO.1-puro expression vectors and the corresponding Mission Lentiviral transduction particles were custom-made by Sigma–Aldrich. The vendor's recommended control particles were also used. HeLa cells were transduced with the viral particles, and puromycin-resistant clones were selected by following the vendor's procedures. The positive clones were selected by measuring heme synthesis rate of the cells. For measurement of heme synthesis, HeLa cells and puromycin-resistant clones were incubated with 0.4 μCi radiolabeled aminolevulinic acid (4-^{14}C, Perkin–Elmer Life Sciences) for 24 h. Heme was extracted from the cells by using acetone–HCl and diethyl ether, and the amount of radiolabeled heme was measured as described previously [30,33]. The incorporation of radioactivity into the extracted heme allows the measurement of heme biosynthesis. The amount of radiolabeled heme was measured by scintillation counting. Measurement of the growth rate of HeLa cells and clones was performed as described previously [29].

2.3. Preparation of extracts and Western blotting analysis

Confirmed HeLa colonies with reduced heme synthesis were grown in heme-depleted medium and then treated with 10 μM heme for various times. Cells were collected, and cell extracts were prepared according to the protocol provided by Cell Signaling Technology. Protein concentrations were measured by using the bicinchoninic acid protein assay kit (Pierce, Rockford, IL). Western blotting analysis was performed as described [29,33]. Polyclonal anti-phospho (Thr202/Tyr204)-MAPK (ERK1/2), anti-ERK1/2, anti-phospho Jak2 (Tyr 1007/1008), anti-Jak2, anti-phospho Src (Tyr-527), anti-Src, and other antibodies were purchased from Cell Signaling Technology.

2.4. Pull-down with hemin-agarose beads

We preformed pull-down with hemin-agarose beads as described previously [34]. Carbonic anhydrase, BSA, and β-amylase were detected by Coomassie blue staining. Jak2 and Src were detected by using Western blotting with the corresponding antibodies. Trypsin digestion of carbonic anhydrase, Jak2, and Src was performed by incubation with various amounts of sequencing grade trypsin (Promega) for 20 min.

3. Results

Using a computational method to identify potential heme-regulated proteins, we found that 5323 proteins of the 20,495 known human proteins in the database contain one or more CP motifs. This represents a large number of proteins for further experimental studies. We therefore decided to study a small group of proteins with crucial functions in intracellular signal transduction. Particularly, by performing GO analysis, we found that 23 non-membrane spanning or non-receptor tyrosine kinases out of 39 known tyrosine kinases (p value: 1.33E−05; $p < 0.000{,}527$ after correction for multiple tests) contain at least one CP motif (Fig. 1A). Because the activity of tyrosine kinases is known to be regulated by the phosphorylation of certain key tyrosine residues, whether heme controls their activity can be determined by examining the effect of heme on the phosphorylation states of such key Tyr residues. Based on the known information about the tyrosine kinases and the availability of specific antibodies to phosphorylated tyrosine kinases, we selected and acquired 10 antibodies specifically to Abl, Ack-1, Jak1, Jak2, Src, and Tyk2 (Fig. 1A), in order to examine the effect of heme on the regulation of these kinases.

To examine the effect of heme on the regulation of tyrosine kinases, we need to use cells that synthesize lower levels of heme than normal cells, or heme-deficient cells [30,35,36]. All mammalian cells require heme for survival and constitutively synthesize heme. Thus, normal cells maintain a level of heme that is necessary for the proper functioning of cellular proteins. To maximize the likelihood of uncovering those proteins that are regulated by heme, we need to lower intracellular heme levels, and then examine the effect of addition of exogenous heme. To this end, we generated stable HeLa cells that exhibit reduced levels of heme synthesis by using the RNAi technique to knock down heme synthetic enzymes.

Measurement of heme synthesis showed that one siRNA for ALAS1 and three for PBGD generated cells with considerably lower levels of heme synthesis compared to untreated HeLa cells or cells expressing a control non-human or mouse siRNA (Fig. 1B). As expected from previous studies using the chemical inhibitor of ALAS, succinyl acetone, knocking down heme synthesis also significantly reduced cell growth rate (Fig. 1C) [29,37]. Evidently the extent of reduction of heme synthesis by siRNAs was less than that caused by a high concentration of succinyl acetone. As such, these HeLa cells with reduced heme synthesis can be maintained for many generations without apoptosis, although they grew at a slower rate [29,37].

To examine the effect of heme on tyrosine kinases, we used the heme-deficient HeLa cell clone PBDG C3, which exhibited the lowest level of heme synthesis and growth rate among the clones (Fig. 1B and C). Still, the effects of heme on ERK1/2, Jak2, and Src were detectable in other clones with reduced heme synthesis. Heme-deficient cells were treated with 10 μM heme for 0, 30 min to 30 h. Then cytoplasmic extracts were prepared from

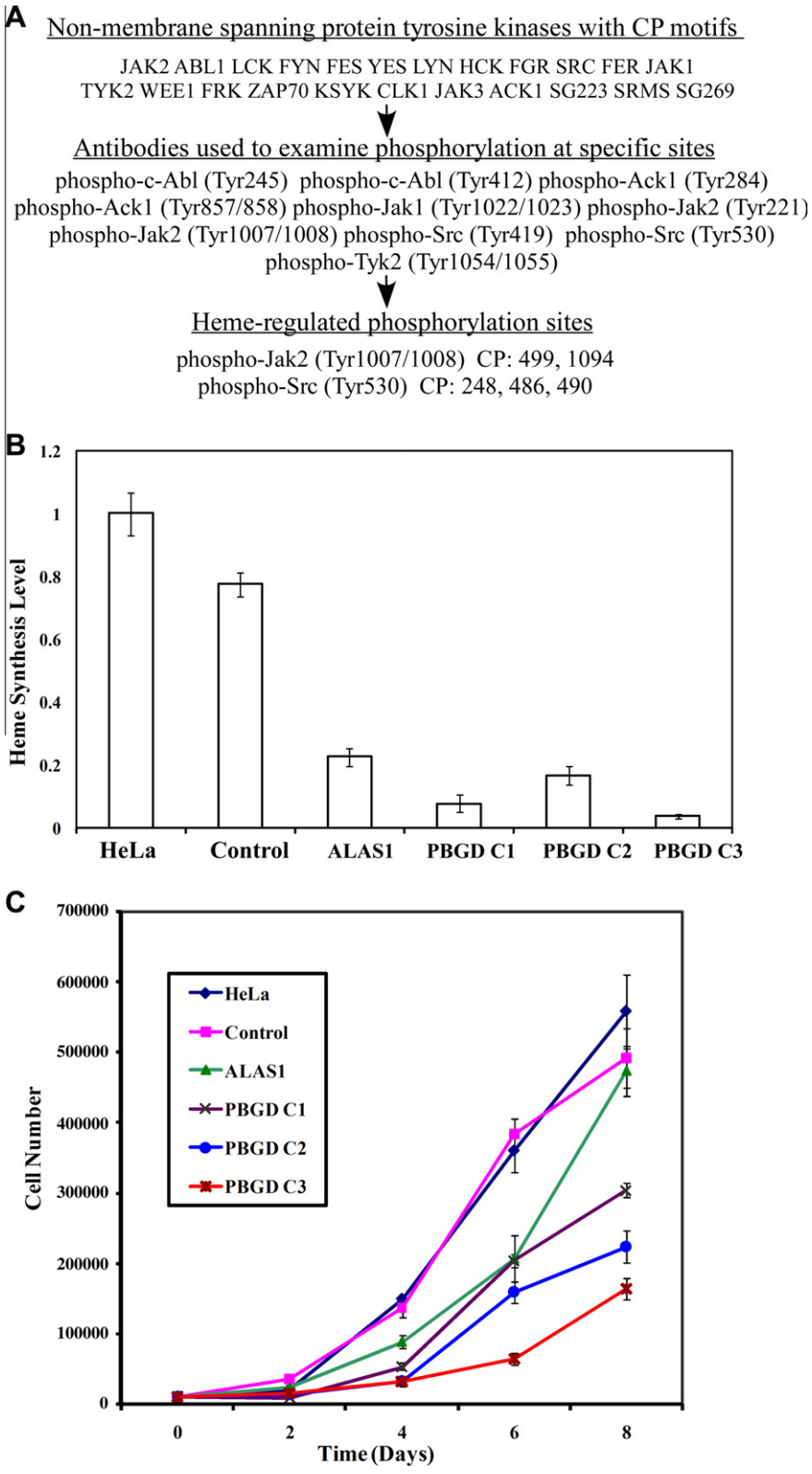

Fig. 1. (A) An outline for identifying heme-regulated tyrosine kinases. A computational search initially identified 23 non-membrane spanning protein tyrosine kinases with the potential heme binding and regulatory CP motifs. Ten commercially available antibodies were then selected to examine whether or not any of the tyrosine kinases is regulated by heme. Eventually two kinases, Jak2 and Src, were found to be affected by heme. (B) Intracellular heme synthesis levels in HeLa cells with heme synthetic enzymes knocked down. Heme was extracted from HeLa cells, control HeLa cells selected from cells transduced with control viral particles, and puromycin-resistant HeLa cell clones transduced with viral particles that allow the expression of shRNA for ALAS1 or PBGD (clones C1 to C3). The levels were calculated as percentages of those of HeLa cells. The plotted data here are averages of at least three replicates. (C) Comparison of the growth of untreated HeLa cells, HeLa cells transduced with the control viral vector, and HeLa clones with the heme synthetic enzyme knocked down. Cells were seeded at 10,000 cells per well, and maintained in DMEM medium with 10% FBS. Cells were collected and counted at the indicated time points. The plotted data here are averages of at least three replicates.

the cells, and were subjected to Western blotting analysis. We used 10 antibodies against various tyrosine kinases phosphorylated at specific residues to examine the states of phosphorylation of different tyrosine kinases that affect their activity (Fig. 1A). Among these antibodies used, only two antibodies consistently detected heme-induced changes (Fig. 2). The rest did not show reliable signal from Western blotting analysis or did not show consistent changes between heme-deficient cells and heme-sufficient cells.

Initially, as a control, we showed that ERK1/2 was phosphorylated/activated soon after the addition of heme (Fig. 2A). This is in complete agreement with previous results [29,37]. Fig. 2B shows that Src is phosphorylated at Tyr530 soon after addition of heme. Phosphorylation at this residue is inhibitory to Src kinase activity [13,38]. Interestingly, heme promotes the phosphorylation of Jak2 at Tyr1007/1008 about 12–30 h post heme addition (Fig. 2C). Jak2 was not phosphorylated at earlier times post heme addition. Phosphorylation at Tyr1007 is required for Jak2 activation [39]. These results clearly show that heme can regulate the phosphorylation of key residues of Jak2 and Src, and thereby control their activity.

Jak2 and Src are key signaling kinases that control many cellular processes [13,40]. We therefore decided to further examine the potential interactions of heme with Jak2 and Src, respectively. To this end, we used a purified active, full-length human Src protein and an active human Jak2 fragment containing residues 808–1132. First, we examined whether Src and Jak2 bind to heme by pull-down using hemin-agarose beads, as described previously [34]. For controls and comparison, we also performed pull-down of BSA, carbonic anhydrase, and β-amylase. BSA is known to bind to heme with high affinity, whereas carbonic anhydrase and β-amylase do not interact with heme. As shown in Fig. 3, the pull-down experiment showed that Jak2 and Src, like BSA, bound to heme, whereas carbonic anhydrase and β-amylase did not, as expected.

Second, we examined whether heme alters the conformation of Jak2 and Src by using protease digestion. We reasoned that if heme interacts with Jak2 and Src, it would likely change their conformation, which may cause changes in protease sensitivity. Indeed, we found that heme altered the sensitivity of Jak2 and Src to trypsin (Fig. 4). As shown in Fig. 4A, in the presence of heme, Src became much more sensitive to trypsin digestion. Likewise, Jak2 also became more sensitive to trypsin in the presence of heme, although to a lesser extent (Fig. 4B). As a control, we showed that heme did not make carbonic anhydrase more sensitive to trypsin. This shows that heme does not have a general effect in enhancing trypsin activity. Together, these results from pull-down and trypsin digestion experiments show that heme interacts actively with Jak2 and Src, and causes conformational changes in Jak2 and Src.

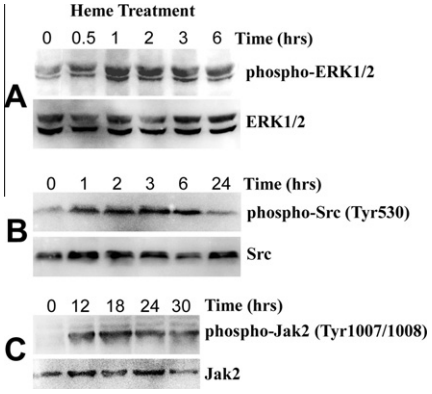

Fig. 2. The effect of heme on the phosphorylation of key residues of ERK1/2 (A), Src (B), and Jak2 (C). HeLa clone PBGD C3 was maintained in heme-depleted medium, and then treated with 10 μM heme for the indicated times. Cells were collected, and protein extracts were prepared and subjected to SDS–PAGE and Western blotting analysis.

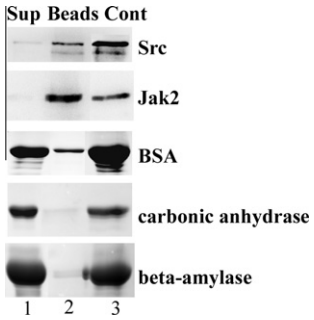

Fig. 3. The pull-down of Src and Jak2 by hemin-agarose beads. Hemin-agarose beads were incubated with purified Jak2 fragment containing residues 808–1132 (C-terminal end), Src, carbonic anhydrase, BSA, and β-amylase. Bound proteins (Beads) and proteins in the supernatant (Sup) were collected and were subjected to SDS–PAGE analysis. Cont = input proteins.

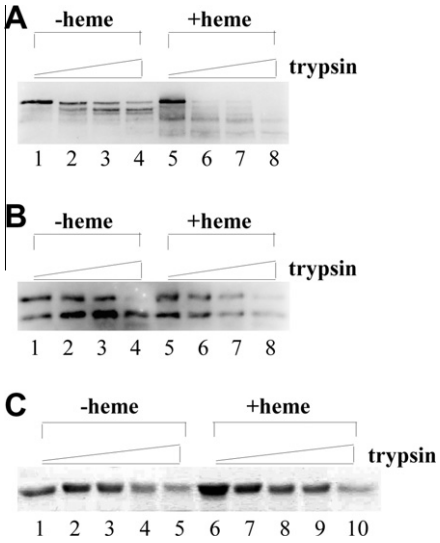

Fig. 4. Heme alters the sensitivity of Jak2 and Src to trypsin digestion. (A) Heme enhances the sensitivity of Src to trypsin. Purified Src was incubated with 0 (lanes 1 and 5), 0.05 (lanes 2 and 6), 0.125 (lanes 3 and 7), and 0.25 ng/μl (lanes 4 and 8) trypsin, in the presence (lanes 5–8) or absence (lanes 1–4) of 10 μM heme. Proteins were analyzed by SDS–PAGE, followed by Western blotting. (B) Heme enhances the sensitivity of Jak2 to trypsin. Purified Jak2 fragment was incubated with 0 (lanes 1 and 5), 0.05 (lanes 2 and 6), 0.125 (lanes 3 and 7), and 0.25 ng/μl (lanes 4 and 8) trypsin, in the presence (lanes 5–8) or absence (lanes 1–4) of 10 μM heme. Proteins were analyzed by SDS–PAGE, followed by Western blotting. (C) Heme does not enhance the sensitivity of carbonic anhydrase to trypsin. Carbonic anhydrase was incubated with 0 (lanes 1 and 5), 2.5 (lanes 2 and 6), 5 (lanes 3 and 7), 12.5 (lanes 4 and 8), and 25 ng/μl (lanes 5 and 10) trypsin, in the presence (lanes 6–10) or absence (lanes 1–5) of 10 μM heme.

4. Discussion

Previous studies have demonstrated that heme is a versatile signaling molecule that can control the activity of diverse cellular regulators [20,21,41]. Such regulators include transcriptional factors Hap1 in yeast, and Bach1, Rev-erbα, and Per2 in mammalian cells [22,34,42–44]. Here we show that heme can control another class of key cellular regulators, tyrosine kinases. This finding has important implications in the studies of heme regulation, tyrosine kinases, and related diseases, such as cancer. It raises the possibility that the activity of tyrosine kinases can be controlled by altering intracellular heme concentration, or by using heme analogues with altered

tyrosine kinase interactions. Further studies to understand the molecular interactions between heme and Jak2 and Src may provide novel insights into the molecular mechanisms by which their activity can be regulated and how drugs targeting Jak2 and Src may be designed.

Our results show that full-length Src and the truncated Jak2 fragment containing residues 808–1132 can bind to heme directly. The Jak2 fragment contains one CP motif at residue 1094 adjacent to Tyr1007. This motif may mediate heme binding to Jak2. However, it is also possible that other residues are involved in heme binding. In the cases of Hap1, Bach1 and Per2, it has been shown that one or more CP motifs mediate heme binding and heme activation of these proteins [24,31,32]. In the case of Rev-erbα, a His residue is essential for heme binding and heme regulation [45]. Further studies are necessary to determine the exact residues involved in heme binding to Jak2. Intriguingly, the Jak2 fragment is phosphorylated and activated. This raises the possibility that heme is not only required for the phosphorylation of Tyr1007 and the activation of Jak2, but it is also needed for the maintenance and continued activation of Jak2 kinase activity.

The phosphorylation and regulation of Src activity are well understood [13,38]. Src is controlled by the phosphorylation of two key Tyr residues, Tyr419 and Tyr530. The phosphorylation of Tyr419 is important for the full activation of Src, whereas the phosphorylation of Tyr530 is inhibitory and renders Src to adopt an inactive conformation [13,38]. In vivo, the phosphorylation of Tyr530 is clearly enhanced by heme during the early times of heme addition (Fig. 2B), while we did not observe any detectable effect of heme on the phosphorylation of Tyr419. Src contains three CP motifs at residues 248, 486, and 490 (Fig. 1A). Residues 486 and 490 are proximal to the Tyr phosphorylation sites, and may mediate heme binding. Other residues beside the CP motifs may also be important for interaction with heme. Further studies are necessary to elucidate the detailed events underlying heme interaction. Nonetheless, our results clearly show that heme can bind to and regulate the activity of two important tyrosine kinases Src and Jak2. These results provide a molecular basis for further studies to investigate the molecular mechanisms by which heme regulates the activity of Src and Jak2 and to identify novel ways of controlling their activity.

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References

[1] J. Schlessinger, M.A. Lemmon, SH2 and PTB domains in tyrosine kinase signaling, Sci. STKE (2003) RE12.
[2] R. Perona, Cell signalling: growth factors and tyrosine kinase receptors, Clin. Transl. Oncol. 8 (2006) 77–82.
[3] A. Csiszar, Structural and functional diversity of adaptor proteins involved in tyrosine kinase signalling, Bioessays 28 (2006) 465–479.
[4] P. Filippakopoulos, S. Muller, S. Knapp, SH2 domains: modulators of nonreceptor tyrosine kinase activity, Curr. Opin. Struct. Biol. 19 (2009) 643–649.
[5] Y. Tohyama, H. Yamamura, Protein tyrosine kinase, syk: a key player in phagocytic cells, J. Biochem. 145 (2009) 267–273.
[6] A.M. Gilfillan, J. Rivera, The tyrosine kinase network regulating mast cell activation, Immunol. Rev. 228 (2009) 149–169.
[7] A. Mocsai, J. Ruland, V.L. Tybulewicz, The SYK tyrosine kinase: a crucial player in diverse biological functions, Nat. Rev. Immunol. 10 (2010) 387–402.
[8] E.K. Yim, S. Siwko, S.Y. Lin, Exploring Rak tyrosine kinase function in breast cancer, Cell Cycle 8 (2009) 2360–2364.
[9] D.L. Stirewalt, S. Meshinchi, Receptor tyrosine kinase alterations in AML – biology and therapy, Cancer Treat Res. 145 (2009) 85–108.
[10] P.K. Wagh, B.E. Peace, S.E. Waltz, Met-related receptor tyrosine kinase Ron in tumor growth and metastasis, Adv. Cancer Res. 100 (2008) 1–33.
[11] L.V. Sequist, T.J. Lynch, EGFR tyrosine kinase inhibitors in lung cancer: an evolving story, Annu. Rev. Med. 59 (2008) 429–442.
[12] J. Lin, R. Arlinghaus, Activated c-Abl tyrosine kinase in malignant solid tumors, Oncogene 27 (2008) 4385–4391.
[13] M. Guarino, Src signaling in cancer invasion, J. Cell Physiol. 223 (2010) 14–26.
[14] K. Fizazi, The role of Src in prostate cancer, Ann. Oncol. 18 (2007) 1765–1773.
[15] S. Li, Src kinase signaling in leukaemia, Int. J. Biochem. Cell Biol. 39 (2007) 1483–1488.
[16] A. Verma, S. Kambhampati, S. Parmar, L.C. Platanias, Jak family of kinases in cancer, Cancer Metastasis Rev. 22 (2003) 423–434.
[17] S.N. Constantinescu, M. Girardot, C. Pecquet, Mining for JAK–STAT mutations in cancer, Trends Biochem. Sci. 33 (2008) 122–131.
[18] L. Valentino, J. Pierre, JAK/STAT signal transduction: regulators and implication in hematological malignancies, Biochem. Pharmacol. 71 (2006) 713–721.
[19] P.R. Ortiz de Montellano, Hemes in Biology, Wiley Encyclopedia of Chemical Biology, John Wiley & Sons, Ltd, West Sussex, 2009. pp. 240–249.
[20] S.M. Mense, L. Zhang, Heme: a versatile signaling molecule controlling the activities of diverse regulators ranging from transcription factors to MAP kinases, Cell Res. 16 (2006) 681–692.
[21] S. Hou, M.F. Reynolds, F.T. Horrigan, S.H. Heinemann, T. Hoshi, Reversible binding of heme to proteins in cellular signal transduction, Acc. Chem. Res. 39 (2006) 918–924.
[22] L. Yin, N. Wu, J.C. Curtin, M. Qatanani, N.R. Szwergold, R.A. Reid, G.M. Waitt, D.J. Parks, K.H. Pearce, G.B. Wisely, M.A. Lazar, Rev-erbalpha, a heme sensor that coordinates metabolic and circadian pathways, Science 318 (2007) 1786–1789.
[23] L. Zhang, L. Guarente, Heme binds to a short sequence that serves a regulatory function in diverse proteins, EMBO J. 14 (1995) 313–320.
[24] S. Hira, T. Tomita, T. Matsui, K. Igarashi, M. Ikeda-Saito, Bach1, a heme-dependent transcription factor, reveals presence of multiple heme binding sites with distinct coordination structure, IUBMB Life 59 (2007) 542–551.
[25] S. Liu, S. Bhattacharya, A. Han, R.N. Suragani, W. Zhao, R.C. Fry, J.J. Chen, Haem-regulated eIF2alpha kinase is necessary for adaptive gene expression in erythroid precursors under the stress of iron deficiency, Br. J. Haematol. 143 (2008) 129–137.
[26] B.G. Yun, J.A. Matts, R.L. Matts, Interdomain interactions regulate the activation of the heme-regulated eIF 2 alpha kinase, Biochim. Biophys. Acta 1725 (2005) 174–181.
[27] J. Igarashi, M. Murase, A. Iizuka, F. Pichierri, M. Martinkova, T. Shimizu, Elucidation of the heme binding site of heme-regulated eukaryotic initiation factor 2alpha kinase and the role of the regulatory motif in heme sensing by spectroscopic and catalytic studies of mutant proteins, J. Biol. Chem. 283 (2008) 18782–18791.
[28] M. Faller, M. Matsunaga, S. Yin, J.A. Loo, F. Guo, Heme is involved in microRNA processing, Nat. Struct. Mol. Biol. 14 (2007) 23–29.
[29] W. Ye, L. Zhang, Heme controls the expression of cell cycle regulators and cell growth in HeLa cells, Biochem. Biophys. Res. Commun. 315 (2004) 546–554.
[30] Y. Zhu, T. Hon, W. Ye, L. Zhang, Heme deficiency interferes with the Ras-mitogen-activated protein kinase signaling pathway and expression of a subset of neuronal genes, Cell Growth Differ. 13 (2002) 431–439.
[31] A. Hach, T. Hon, L. Zhang, A new class of repression modules is critical for heme regulation of the yeast transcriptional activator Hap1, Mol. Cell. Biol. 19 (1999) 4324–4333.
[32] T. Hon, A. Hach, H.C. Lee, T. Cheng, L. Zhang, Functional analysis of heme regulatory elements of the transcriptional activator Hap1, Biochem. Biophys. Res. Commun. 273 (2000) 584–591.
[33] A. Sengupta, T. Hon, L. Zhang, Heme deficiency suppresses the expression of key neuronal genes and causes neuronal cell death, Brain Res. Mol. Brain Res. 137 (2005) 23–30.
[34] J. Yang, K.D. Kim, A. Lucas, K.E. Drahos, C.S. Santos, S.P. Mury, D.G. Capelluto, C.V. Finkielstein, A novel heme-regulatory motif mediates heme-dependent degradation of the circadian factor period 2, Mol. Cell. Biol. 28 (2008) 4697–4711.
[35] Y. Zhu, T. Hon, L. Zhang, Heme initiates changes in the expression of a wide array of genes during the early erythroid differentiation stage, Biochem. Biophys. Res. Commun. 258 (1999) 87–93.
[36] Y. Zhu, H.C. Lee, L. Zhang, An examination of heme action in gene expression: heme and heme deficiency affect the expression of diverse genes in erythroid k562 and neuronal PC12 cells, DNA Cell Biol. 21 (2002) 333–346.
[37] W. Ye, L. Zhang, Heme deficiency causes apoptosis but does not increase ROS generation in HeLa cells, Biochem. Biophys. Res. Commun. 319 (2004) 1065–1071.
[38] J.M. Bradshaw, The Src, Syk, and Tec family kinases: distinct types of molecular switches, Cell Signal. 22 (2010) 1175–1184.
[39] J. Feng, B.A. Witthuhn, T. Matsuda, F. Kohlhuber, I.M. Kerr, J.N. Ihle, Activation of Jak2 catalytic activity requires phosphorylation of Y1007 in the kinase activation loop, Mol. Cell. Biol. 17 (1997) 2497–2501.
[40] M. Kurdi, G.W. Booz, JAK redux: a second look at the regulation and role of JAKs in the heart, Am. J. Physiol. Heart Circ. Physiol. 297 (2009) H1545–H1556.
[41] A.S. Tsiftsoglou, A.I. Tsamadou, L.C. Papadopoulou, Heme as key regulator of major mammalian cellular functions: molecular, cellular, and pharmacological aspects, Pharmacol Ther. 111 (2006) 327–345.
[42] L. Zhang, A. Hach, Molecular mechanism of heme signaling in yeast: the transcriptional activator Hap1 serves as the key mediator, Cell. Mol. Life Sci. 56 (1999) 415–426.
[43] K. Ogawa, J. Sun, S. Taketani, O. Nakajima, C. Nishitani, S. Sassa, N. Hayashi, M. Yamamoto, S. Shibahara, H. Fujita, K. Igarashi, Heme mediates derepression of Maf recognition element through direct binding to transcription repressor Bach1, EMBO J. 20 (2001) 2835–2843.

[44] K. Kitanishi, J. Igarashi, K. Hayasaka, N. Hikage, I. Saiful, S. Yamauchi, T. Uchida, K. Ishimori, T. Shimizu, Heme-binding characteristics of the isolated PAS-A domain of mouse Per2, a transcriptional regulatory factor associated with circadian rhythms, Biochemistry 47 (2008) 6157–6168.

[45] S. Raghuram, K.R. Stayrook, P. Huang, P.M. Rogers, A.K. Nosie, D.B. McClure, L.L. Burris, S. Khorasanizadeh, T.P. Burris, F. Rastinejad, Identification of heme as the ligand for the orphan nuclear receptors REV-ERBalpha and REV-ERBbeta, Nat. Struct. Mol. Biol. 14 (2007) 1207–1213.