 doi:10.20944/preprints202101.0626.v1

Review

A novel therapeutic target, BACH1, regulates cancer metabolism

Joselyn Padilla [1] and Jiyoung Lee [1]*

[1] Department of Biochemistry and Molecular Medicine, School of Medicine & Health Sciences, George Washington University, Washington, DC 20037

* Correspondence: jiyounglee@gwu.com; Tel.: +1-202-994-2284

Abstract: BTB domain and CNC homology 1 (BACH1) is a highly expressed transcription factor in tumors including breast and lung, relative to their non-tumor tissues. BACH1 is known to regulate multiple physiological processes including heme homeostasis, oxidative stress response, senescence, cell cycle, and mitosis. In a tumor, BACH1 promotes invasion and metastasis of cancer cells, and the expression of BACH1 presents a poor outcome for cancer patients including breast cancer patients. Recent studies identified novel functional roles of BACH1 in the regulation of metabolic pathways in cancer cells. BACH1 inhibits mitochondrial metabolism through transcriptional suppression of mitochondrial membrane genes. In addition, BACH1 suppresses activity of pyruvate dehydrogenase (PDH), a key enzyme that converts pyruvate to acetyl-CoA for the citric acid (TCA) cycle through transcriptional activation of pyruvate dehydrogenase kinase (PDK). Moreover, BACH1 increases glucose uptake and lactate secretion in aerobic glycolysis through the expression of metabolic enzymes involved such as hexokinase 2 (HK2) and glyceraldehyde 3- phosphate dehydrogenase (GAPDH). Pharmacological or genetic inhibition of BACH1 could reprogramme metabolic pathways, subsequently rendering metabolic vulnerability of cancer cells. Furthermore, inhibition of BACH1 decreased antioxidant-induced glycolysis rates as well as reduced migration and invasion of cancer cells, suggesting BACH1 as a potentially useful cancer therapeutic target.

Keywords: BTB and CNC homology 1 BACH1, mitochondrial metabolism, glycolysis, heme oxygenase 1 (HMOX1), mitochondrial electron transport chain (ETC), Nrf2 (encoded by Nfe2l2), metformin, hemin, breast cancer, lung cancer

1. Introduction

Metabolism is an essential process to acquire necessary nutrients from outside of cells and utilize them in their biomass to maintain cell viability. In a tumor microenvironment that has limited nutrients and oxygen, nutrient availability and nutritional stresses often rewire metabolic pathways of cancer cells. Hence, cancer cells exhibit distinct metabolic phenotypes to support their survival and proliferation states in tumor microenvironment [1]. The most common metabolic phenotypic changes in cancer cells are increased glucose uptake for aerobic glycolysis and lactate production even in the presence of oxygen [2]. In response to the metabolic stress or oncogenic signals, cancer cells also utilize alternative carbon and energy sources such as fatty acids and amino acids, including glutamine, using micropinocytosis to fulfill increased energy demands [3–6]. Due to the distinct cancer metabolism, blocking primary metabolic pathways of cancer cells generates a cancer vulnerability by effectively reducing proliferation of cancer cells or inhibiting survival of cancer cells. Currently, several drugs targeting altered and activated metabolic pathways in cancer cells have been approved, or are under current clinical trials as a potential cancer treatment [7]. However, inhibition of particular metabolic pathways can be ineffective or less effective, because cancer cells have the metabolic flexibility to use different substrates or alternative metabolic pathways to adapt various resources for their survival [8, 9]. Thus rewiring their metabolism is crucial to make cancer cells more targetable and susceptible.

 doi:10.20944/preprints202101.0626.v1

It is fundamentally important to understand cancer metabolism to achieve a more effective treatment, as well as, to identify accurate prognostic and predictive biomarkers for cancer patients. Recently, several studies identified a new functional role of BACH1 in cancer metabolism regulation. BACH1 works as a metabolic driver in response to the intracellular and extracellular signals in breast and lung cancer cells [10–12]. This review highlights recent advances in understanding of the essential role of BACH1 on cancer metabolism and its potential as a therapeutic target for cancer therapy. Of note, Bach1 plays critical roles for other physiological process regulation including cellular senescence independent of p53, spindle orientation rearrangement during mitosis upon phosphorylation on Bach1, and myocardiac function against stress but those are not discuss in this review [13-16].

2. Heme-binding transcription factor, BACH1

The BTB domain and CNC homology (BACH), is a member of the Cap 'n' Collar (CNC)/pox virus and zinc finger (POZ) protein that contains a basic leucine zipper (bZIP) domain and a Bric-a-brac-Tramtrack-Broad complex (BTB) domain at the N-terminal region [17]. Two BACH family protein members, BACH1 and BACH2, have similar sequences and structures containing a BTB and bZIP domain, and heme binding motifs [17]. Both BACH1 and BACH2 are widely expressed in most human tissues. BACH1 mRNA is abundantly enriched in cell types such as neutrophils, NK cells, monocytes, macrophages, and dendritic cells, while BACH2 mRNA in B cells and T cells implying non-redundant functions of BACH1 and BACH2 [18]. As a member of bZIP transcription factor family, the BACH protein makes a dimer (either homodimer or heterodimer) with other transcription factors, which give them regulatory agility for transcription activity [19]. The bZIP domain allows BACH1 to recognize the DNA elements known as MAF recognition elements (MAREs), which is well conserved both in human and mouse, forming a well characterized heterodimer with small MAF proteins including MAFF, MAFG, and MAFK [13], [20].

One feature of the BACH protein is the cytoplasmic localization signal (CLS) both in BACH1 and BACH2. A truncated mutation of the CLS domain in BACH1 protein abolished cytoplasmic localization keeping BACH1 proteins only in the nucleus. Distinct functional roles of BACH proteins in innate immunity and BACH2 in adaptive immune systems are well documented in other review papers [13]. Another feature of BACH proteins is that the multiple heme-binding cysteine-proline (CP) motifs are located in 6 different sites at the C-terminal regions in BACH1, and in 5 different sites in BACH2 [15, 18]. Biochemical assays using a mutation on cysteine (C) and proline (P) residues in BACH1 showed that BACH proteins directly interact with free heme. Heme-bound BACH1 proteins undergo proteasomal degradation after nuclear export.

As a heme-binding transcription factor, BACH1 was revealed to be a heme homeostasis regulator sensing intracellular heme levels [21]. Heme is a compound of porphyrin class that contains an iron in the middle of structure. Heme plays important regulatory roles in cells and provides a prosthetic group for many heme proteins including hemoglobin and cytochrome *c* oxidase [22]. Glutathione-S-transferase (GST)-fused Bach1 derivatives showed heme affinity *in vitro* and the mutation of CP motifs in Bach1 interrupted heme interactions. Upon heme-binding, BACH1 unbinds DNA and undergoes nuclear export for ubiquitin-dependent degradation, thus releasing transcription suppression of BACH1 target genes including heme oxygenase 1 (*HMOX1* or *HO-1*) [23, 24]. Simultaneously, accumulated Nrf2 (encoded by *NFE2L2*) replaces BACH1 forming a heterodimer with Maf proteins for transcriptional activation of target genes such as *HMOX1* mRNA (**Figure 1a**). Heme-inducible and BACH1-mediated *HMOX1* expression further catabolizes heme into biliverdin and CO, releasing Fe^{2+} [23]. Consequently, BACH1 controls heme levels by regulating expression of HMOX1, generating a negative feedback loop.

In lung cancer cells that were initially isolated from the genetically engineered mice ($Kras^{G12D/+}$;$p53^{-/-}$), keap1 loss (or Nrf2 activation) using sgKeap1 stabilizes Bach1 stability through *Hmox1* expression. These mechanisms clearly demonstrated that ubiquitin ligase Fbxo22 is involved in heme-dependent degradation of Bach1 [12]. In this study, the molecular mechanisms indicate that heme promotes the physical interaction between Bach1 and Fbxo22 in lung cancer cells. Alanine scanning assays could map Fbxo22 interaction motif with Bach1. They showed a failed interaction of Bach1 with Fbxo22 when Bach1 residues at 9 phenylalanine (F9A), 11 tyrosine (Y11A), and 13 serine (S13A) were mutated. Furthermore, in vitro studies based on cell lines overexpressing wild type Bach1 or mutant Bach1 (Y11F) validated that mut Bach1 (Y11F) is more stable than WT Bach1 in abundant heme condition. Activated Nrf2 which is released from Keap1 mutation or Keap1 loss replaces Bach1 sites for transactivation for heme homeostasis, and also stabilizes Bach1 in a Hmox1-dependent manner [21, 23].

As a transcriptional factor, genome-wide BACH1 bindings were validated using a chromatin immunoprecipitation combined with deep sequencing (ChIP-seq) assay [25, 26]. ChIP-seq analyses validated target genes such as heme oxygenase 1 (*HMOX1*) and glutamate-cysteine ligase modifier/catalytic subunit (*GCLM*/*GCLC*) involved in heme degradation and redox regulation, respectively [25]. Also the analyses of ChIP-seq data uncovered previously unidentified BACH1 binding regions involved in the functional pathways such as cell cycle, apoptosis, and cell proliferation. Another study performing BACH1 ChIP-seq using a primary mouse embryonic fibroblast (MEF) identified peroxisome proliferator -activated receptor gamma (*Pparg*), a key factor of adipogenesis related to lipid metabolism, as a novel Bach1 target gene in MEFs [26]. It was suggested that BACH1 may have other binding partners other than MAF proteins when BACH1 bindings were compared with MAFK bindings using a MAFK ChIP-seq dataset in their studies. Analyses of BACH1 ChIP-seq indicate that BACH1 mostly binds to the DNA element independent of MAFK protein because bindings of BACH1 overlapped only about 11% with those of MAFK on the whole genome. Also, noticeable differences were observed between BACH1 motifs and MAFK motifs identified in their ChIP-seq data set. However, it is not fully understood yet whether BACH1 has different partner proteins or binding cofactors to form oligomers or works as a monomer or homodimer for the transactivation of particular sets of target gene transcripts. It is noteworthy that BACH1 regulatory roles might be tissue-, cell type-, or species-specific.

3. BACH1 regulates cancer metastasis

BACH1 is expressed aberrantly higher in tumor tissues than non-tumor control tissues. Breast tumors express more *BACH1* mRNA than naïve breast tissues. In particular, basal like-breast cancer or triple negative breast cancer (TNBC) subtype displays distinctive levels of *BACH1* transcripts when compared with other breast cancer subtypes such as luminal A, luminal B, or her2-positive subtypes [10, 27]. Elevated expression levels of *BACH1* in cancer are mainly due to genetic amplification, found in breast cancer, or increased protein stability found in lung cancer [6-8, 20]. The cancer cells enriched with BACH1 showed promoted metastatic properties such as migration, invasion, intravasation and metastasis of cancer cells both *in vivo* and *in vitro*. Thereby *BACH1* suppression resulted in reduction of each stage of the metastatic processes such as migration, invasion, and micrometastasis [11, 12, 28–30]. In breast cancer cells, BACH1 enhanced expression of matrix metalloproteinases (*MMPs)* and CXC-chemokine receptor 4 (*CXCR4*) mRNA as a transcriptional activator through direct interaction on the promoter regions of target genes to promote metastatic progression, without affecting primary breast tumor growth [28, 29]. Therefore, a gene signature that consists of *BACH1* and its target genes including *MMP1* and *CXCR4* gives a worse prognosis for patients with TNBC indicated by shorter metastasis-free survival (MFS) rates within 5 years when analyzed in patient data cohorts (n=878 and n=470) [27].

 doi:10.20944/preprints202101.0626.v1

Moreover, either antioxidant treatment-induced BACH1 or Keap1 loss-induced Bach1 could promote metastasis in lung tumor models [11, 12]. Blockade of glycolysis pathways significantly reduced metastasis phenotypes driven by Bach1 in lung cancer. Inhibition of Bach1 target gene, HMOX1, or Bach1-ligase overexpression could reduce Bach1-driven metastasis of lung cancer. In addition, BACH1 promotes metastatic processes in pancreatic ductal adenocarcinoma (PDAC). Thus BACH1 silencing decreased metastasis of pancreas cancer cells, both *in vitro* and *in vivo*, by inducing expression of a group of genes involved in epithelial to mesenchymal transition (EMT) process [30].

In addition to transcriptional regulation, BACH1 is also involved in epigenetic regulation in cancer cells [31, 32]. In BRAF(V600E) mutant colon and skin cancer, BACH1 is involved with a complex with MAFG to mediate the function of DNA methyltransferase (DNMT3B) for hypermethylation of their target promoters inducing cancer progression [31].

Consistently, a transcriptional signature that includes Bach1 and its target genes showed strong association with poor survival, advanced clinical stage and grade, and metastasis in lung cancer patients [12]. Expectedly, BACH1 expression showed a positive correlation with poor prognosis in patients with kidney clear carcinoma or pancreatic adenocarcinoma, as well as with lung tumors [11]. Therefore, targeting BACH1 in cancer to suppress its levels is beneficial to patients since BACH1 suppression could reduce tumor metastasis and increase metastasis-free survival of cancer patients. These suggest that BACH1 is a prospective target to treat metastatic tumors.

4. BACH1 regulation in cancer cells

BACH1 expression is regulated at translational levels in cancer cells. In breast cancer cells, a metastasis suppressor, Ras Kinase Inhibitory Protein (RKIP), indirectly suppresses BACH1 expression through a regulatory cascade that includes MAP Kinase, LIN28, and its downstream *let-7* [28, 33]. It was revealed that BACH1 acts as a transcriptional suppressor of RKIP by binding on the upstream promoter of RKIP. Thus RKIP and BACH1 create a mutually suppressive regulatory loop in breast cancer cells [8]. BACH1 promotes cancer metastasis and RKIP suppresses cancer metastasis, showing two molecules with opposite functions suppress each other. The mutually suppressive regulatory network between BACH1 and RKIP represents bi-stability, indicating a tight balance between BACH1 and RKIP expression is needed to coordinate metastasis process of cancer cells. Additionally, BACH1 suppresses its own expression on the BACH1 promoter. Luciferase assays using upstream regions (+1130) from the transcription start site (TSS) of BACH1 showed reduced luciferase activity with BACH1 overexpression or increased luciferase activity with siRNA transfection for BACH1. BACH1 recruitment on the binding regions of the BACH1 promoter was validated through ChIP assays indicating transcriptional regulation on its own expression [34]. These suggest that cellular levels of BACH1 are also tightly regulated in human breast cancer cells.

Another regulatory mechanism of BACH1 expression includes mutation in either *Keap1* or *Nfe2l* that comprises about 30% of human lung cancer [12]. In the *Keap1*-mutated lung adenocarcinoma, activated Nrf2 stabilizes Bach1 expression that further promotes invasion and metastasis of lung tumors. Nrf2 is the master antioxidant transcriptional regulator against oxidative stress and is negatively regulated by Keap1, a Cul3-RING ubiquitin ligase, in the cell. Upon oxidative stress, Nrf2 is released from Keap1 and activates transcriptional activity of antioxidant genes to defend the cells against the stress. A recent study shows that accumulated Nrf2 stabilizes Bach1 expression by inducing Hmox1 expression that oxidizes cellular heme in lung cancer. This mechanism involves Fbox22, a ubiquitin ligase, that interacts with amino acids residues of Bach1 for heme-dependent Bach1 degradation. Pharmacological inhibition of Hmox1 using Zn-PPIX could achieve reduced levels of Bach1 expression through heme down regulation (**Figure 1b**).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 January 2021 doi:10.20944/preprints202101.0626.v1

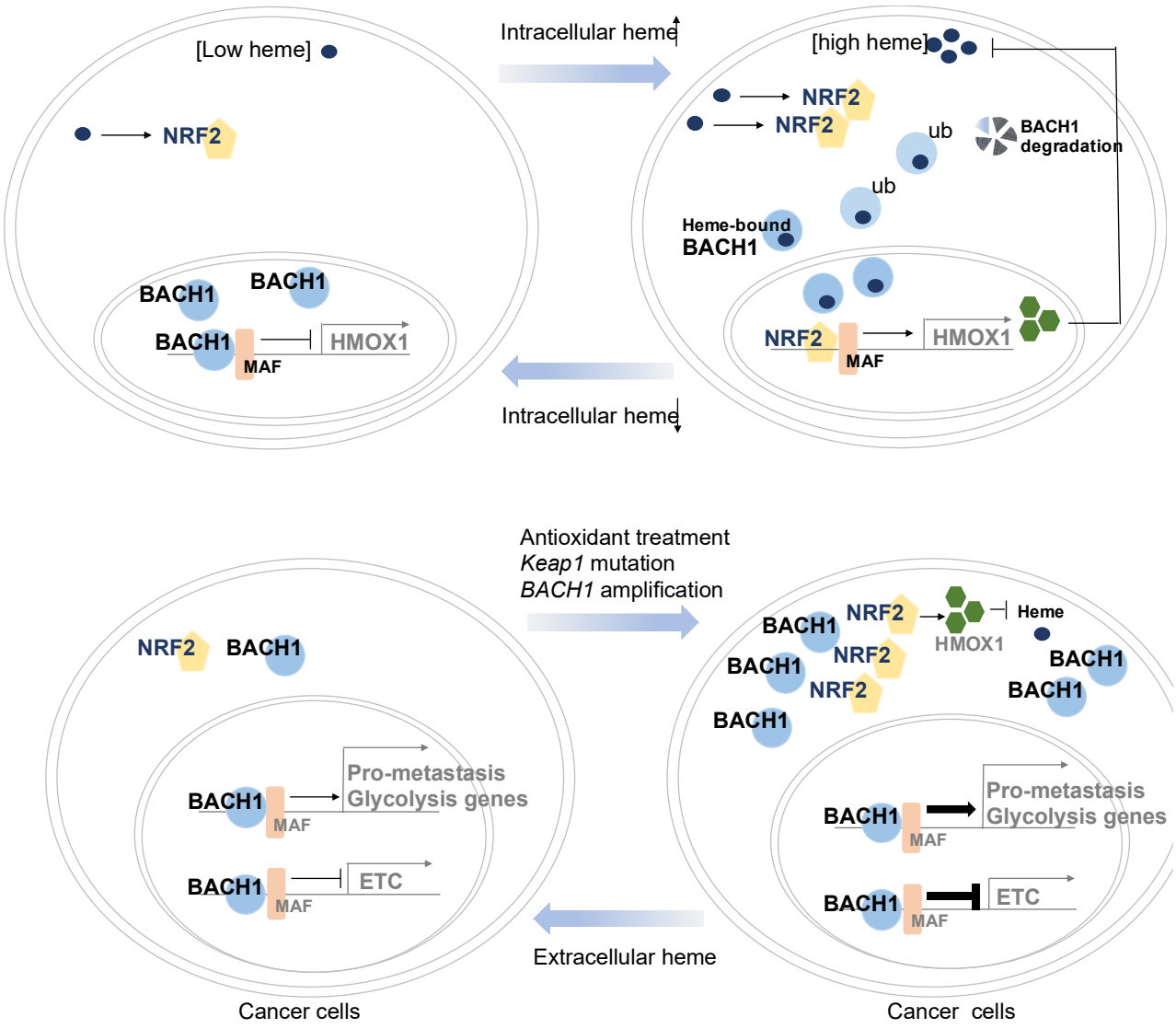

Figure 1. BACH1 regulates genes involved in heme homeostasis and metabolic pathways. (**a**) Heme homeostasis regulation by BACH1. BACH1 and NRF2 interplay for intercellular heme homeostasis. Low heme levels in cells enable BACH1 forming a heterodimer with MAF proteins for transcriptional suppression of heme oxygenase (HMOX1 or HO1). Upon increase of heme levels that are synthesized in mitochondria or by extracellular treatment, activated NRF2 partners with MAF proteins for transactivation of HMOX1. Heme binds to BACH1 and heme-bound BACH1 undergoes nuclear export and further ubiquitin-dependent degradation. Transactivated HMOX1 then suppress heme levels to maintain heme levels by reducing extra heme in cells.; (**b**) Metabolic regulation by BACH1 at transcriptional level. BACH1 suppresses transcriptional activity of genes involved in mitochondrial ETC genes, while BACH1 activates pro-metastatic genes including CXCR4 and MMP1, and genes involved in glycolysis including HK2 and GAPDH. (Abbreviations: BTB and CNC Homology 1; BACH1, NRF2; NFE2N2, Heme oxygenase 1; HMOX1, ub; Polyubiquitination, Electron Transport Chain; ETC.

Furthermore, antioxidant treatment such as N-acetylcysteine (NAC) or vitamin E could stabilize Bach1 expression [11]. Lung cancer cell lines which were isolated and established from the Kras mutant mice that were treated with antioxidants showed reduced ROS levels and stabilized Bach1 protein levels through reducing heme levels, although BACH1 transcripts were not altered. These cells harbored increased invasiveness.

MicroRNA (miRNA) is a small non-coding RNA fragment consisting of about 22 nucleotides for negative regulation of target genes by interacting with the 3′ untranslated region (UTR) regions of genes [35]. BACH1 is also regulated by miRNA-155 or miRNA-330 for proliferation and migration of renal cancer cells or colon cancer [36, 37]. Likewise, Bach1 is regulated by numerous factors in non-tumor tissues. Hypoxia inducing-miR-532-5p also interacts with 3″ UTR of BACH1 to regulate muscular pericyte function including endothelial permeability, vascular stability, and angiogenesis [38]. In lung development, Bach1 expression was regulated by miRNA-196a expression [33]. Transient Bach1 induction was also observed in acute liver injury [40]. Hepatic injury by carbon tetrachloride (CCl_4) demonstrated significant induction of Bach1 mRNA levels in rat animal models. Notably, hypoxia is known to induce *BACH1* transcripts, although it is unclear whether *BACH1* mRNA induction is mediated by HIFs [18].

5. Pharmacological inhibition of BACH1

The heme regulatory role of BACH1 supports that BACH1 protein can be leveraged through excessive free heme (or hemin) as a pharmacological tool. Hemin (Haemin) is an iron-containing porphyrin (Iron-protoporphyrin IX) that is from a heme group [41]. As an essential cofactor in cellular processes, hemin plays important roles for numerous biological functions including oxidative stress response [42]. Injection of hemin is currently in a form of drug (Panhematin), which is approved by the Food and Drug Administration (FDA) to treat patients with acute porphyria. In a previous report, hemin treatment to breast cancer cells and the mice bearing breast tumors exhibited substantially reduced BACH1 levels with unnoticeable toxicity, suggesting hemin as a potentially non-toxic BACH1-specific drug for tumors [10]. Importantly, hemin specificity for BACH1 as a BACH1-drug was tested using a heme-resistant murine mutant Bach1, which has cysteine (C) to alanine (A) point mutations in four of the heme-binding motifs that are required for heme binding, release of BACH1 from DNA for nuclear export and subsequent degradation of BACH1 [10, 21, 43]. Mut Bach1-expressing TNBC cells were resistant to hemin treatment but rescued BACH1 function for gene regulation, metabolic properties, and metformin sensitivity, suggesting hemin acts in the specific degradation of BACH1.

In addition to heme, cadmium is known to induce nuclear export of Bach1, inducing *HMOX1* expression in response to oxidative stress of cells [43]. The CLS domain conserved at the C-terminal of Bach1 and Bach2 is responsible for cadmium response. Based on the fact that cadmium activates p38 and ERK1/2 pathways for Nrf2 activity, Bach1 is also regulated by p38 and ERK1/2 signaling. When cells were treated with a MEK1/2 inhibitor, Bach1 remained in the nucleus in the presence of cadmium, whereas treatment of a p38 inhibitor did not affect Bach1 localization. Yet the authors also suggest that cadmium may inactivate Bach1 through another mechanism in addition to nuclear export. Moreover, a heme-related molecule, Fe^{2+} mesoporphyrin IX (FeMePIX), is able to interact with Bach1 to block DNA binding of Bach1, which further indicates FeMePIX as a potential cellular inhibitor for transactivation of BACH1 [21].

Likewise, zinc(II) mesoporphyrin and cobalt(II) porphyrin are known to contribute up-regulation of HMOX1 mRNA expression through BACH1 degradation in cells [44]. Small inhibitory molecule, HPP-4382, additionally showed induced *HMOX1* mRNA as a BACH1 modulator through the suppression of BACH1 activity as validated by ChIP and reporter assays [45].

6. BACH1 regulates mitochondrial metabolism in cancer

Mitochondrial metabolism provides essential processes for energy metabolism, redox balance, and macromolecule biosynthesis in cancer cells [4]. A large amount of information indicates that intact mitochondria and active mitochondrial metabolism is pivotal for cancer cell survival and proliferation, thus it is suitable to be targeted for anti-cancer therapeutics [46]. Regulatory mechanisms of mitochondrial metabolism in cancer harbor

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 January 2021 doi:10.20944/preprints202101.0626.v1

oncogenic signaling or mutated tumor suppressors, while some mitochondrial metabolites are also oncogenic [4, 47].

Metabolomics, microarray and deep sequencing approaches revealed that metastasis promoting transcriptional factor, BACH1, participates in metabolic reprogramming in cancer cells [8, 27, 29]. Metabolic regulatory function of BACH1 in mouse embryonic fibroblasts (MEFs) was feasibly suggested in Bach1 ChIP-seq analyses [26]. Lipid metabolism such as adipogenesis was detected as a BACH1 target, since peroxisome proliferator-activated receptor gamma (*PPARg*) was observed as a binding target gene of BACH1 in the ChIP-seq results.

In TNBC cells, mitochondrial inner membrane genes are the most significantly changed targets upon BACH1 depletion using shRNA [10, 27]. As a transcriptional factor, BACH1 inhibits transcriptional expression of genes involved in mitochondrial oxidative phosphorylation (OXPHOS) of breast cancer cells as observed by reverse transcriptase quantitative PCR (qRT-PCR) and protein blotting (**Figure 2**). In support of these findings, bioinformatic approaches using KEGG annotation assays presented that mitochondrial OXPHOS is the most inversely functional associated pathway inversely with BACH1 expression in patient tumors including breast, skin, liver, pancreas, and prostate. BACH1 supports aerobic glycolysis by inhibition of mitochondrial electron transport chains (ETC) gene expression in breast tumors. When BACH1 is reduced, expression of ETC genes was de-repressed, thus enhancing mitochondrial respiration of cells as assessed by increasing oxygen consumption rates (OCR) while decreasing extracellular acidification rates (ECAR) of TNBC cells. In addition, the activity of pyruvate dehydrogenase (PDH) that converts pyruvate to acetyl CoA for the entry of the tricarboxylic acid (TCA) cycle was also suppressed by BACH1 through pyruvate dehydrogenase kinase (PDK) regulation. Furthermore, flux analyses using ^{13}C-labeled glucose showed that BACH1 suppresses incorporation of glucose into the TCA cycle intermediates, as metabolomics analyses validated substantially higher levels of glycolysis intermediates and lower levels of the TCA cycle intermediates in the BACH1-enriched TNBC cells relative to the BACH1-depleted cells.

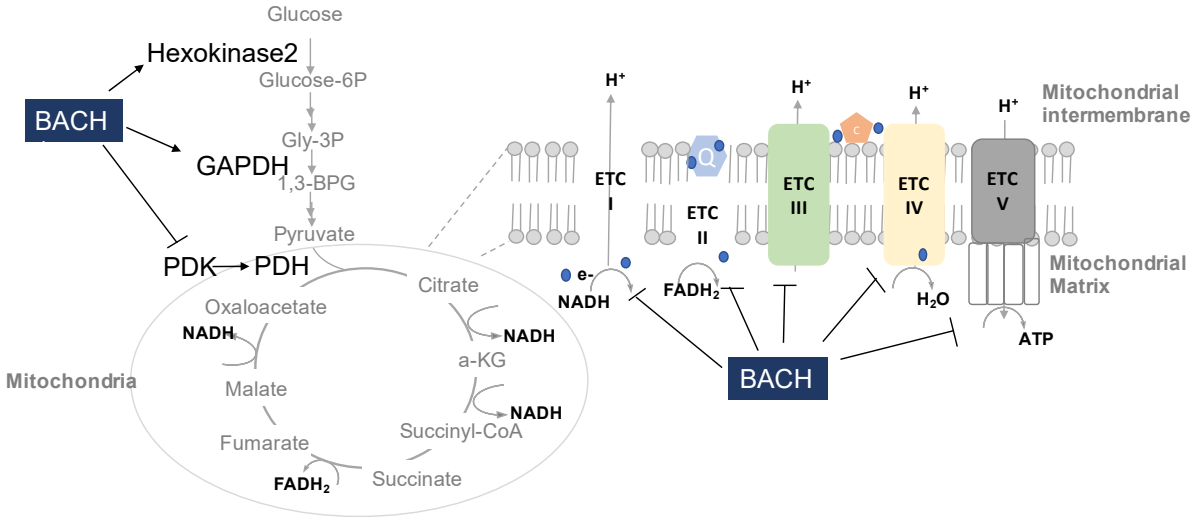

Figure 2. BACH1 regulates metabolic pathways in cancer cells. BACH1 enhances glycolysis pathway by activating expression of *HK2* and *GAPDH mRNA*, while it suppresses mitochondrial metabolism by inhibiting PDH activity through *PDK* regulation and expression of the subunit's genes of mitochondrial ETC complex (I-V) that are coupled to the TCA cycle. (Gly-3P; glyceraldehyde 3-phosphate, 1,3-BPG; 1, 3-bisphosphoglycerate, PEP; phosphenol pyruvate, PDK; pyruvate dehydrogenase kinase, PDH; pyruvate dehydrogenase, aKG; alpha ketoglutarate, HK2; hexokinase, GAPDH; glyceraldehyde 3-phospho dehydrogenase, ETC; electron transport chain, TCA cycle; the citric acid cycle, Q: ubiquinone,

 doi:10.20944/preprints202101.0626.v1

C; cytochrome c, ATP; adenosine triphosphate, NADH; nicotinamide adenine dinucleotide, $FADH_2$; flavin adenine dinucleotide 2)

Since BACH1 regulates the central carbon metabolism in TNBC cells, reducing BACH1 levels either genetically or pharmacologically could rewire metabolic pathways, by enhancing mitochondrial respiration and reducing aerobic glycolysis. The flexible cancer metabolism by BACH1 also plays as a primary metabolic pathway in cancer, thereby generating metabolic liability when primary metabolism is blocked. Subsequently, mitochondrial respiratory inhibitors such as metformin, rotenone, and antimycin A could be even more lethal to the survival of cancer cells that are depleted with BACH1 compared to the BACH1-enriched control in the cell line-based *in vitro* studies. Additionally, transient silencing *COX15* or *UQCRC1* using siRNA in the BACH1-depleted cells as low as the levels of those in control cells completely restored metformin resistance and rescued cell growth. These indicate that mitochondrial OXPHOS plays a critical role for metformin resistance in TNBC cells and is altered by BACH1 manipulation. Notably, BACH1 does not alter the expression of neither metformin transporter (OCT1, encoded by *SLC22A1*) nor mitochondrial biogenesis genes such as PPARg or peroxisome proliferator-activated receptor gamma coactivator1-alpha (PGC1a, encoded by *PPARGC1A*) in TNBC cells.

The addition of pyruvate into the growth media of BACH1-depleted cells rendered drug resistance to metformin treatment. The TNBC cells that are enriched with BACH1 are resistant to metformin treatment regardless of pyruvate, but shBACH1 cells were only resistant to metformin in the presence of pyruvate. The effect of pyruvate was seen in the changes of NAD^+/NADH ratio as previously reported [48]. NAD^+/NADH ratios are important factors in determining metformin responses of cancer cells and were also altered by BACH1 depletion.

In vivo breast tumor mouse models including TNBC patient-derived xenograft (PDX) showed that shBACH1 tumors or BACH1-depleted tumors by hemin injection significantly increased sensitivity of tumors against the treatment with metformin relative to the BACH1-enriched control tumors. This data suggests a novel combination therapeutic strategy using BACH1 suppression and metformin to treat breast tumors. Mouse models bearing mut Bach1-expressing TNBC tumors did not respond to tumor suppression when both metformin and hemin were administered. In support of preclinical studies, bioinformatic analyses of patient tumor expression data from the Cancer Genome Atlas (TCGA) highlighted clinical significance of BACH1 and mitochondrial OXPHOS in numerous cancer types including breast cancer. All together, BACH1 plays a conserved role for mitochondrial metabolism and cancer metabolic phenotypes are reversibly altered by BACH1 levels, suggesting BACH1 as a useful target to generate cancer vulnerability against mitochondrial inhibitors [46].

7. BACH1 regulates redox stress in cancer

Redox regulation is another key physiological function of cells for their survival against oxidative stresses. BACH1 and NRF2 are well known to interplay for cellular redox homeostasis [18]. BACH1 suppresses antioxidant genes by binding to the Antioxidant Response Element (ARE), while NRF2 activates expression of antioxidant genes on the same sites upon receiving stress signals. Under oxidative stress, cells induce free heme by releasing them from heme-containing proteins, which further degrade Bach1 to de-repress antioxidant gene expressions that are suppressed by Bach1 [24]. In this mechanism, oxidative stress accumulates NRF2 proteins for transactivation of antioxidant genes including HMOX1 that further alleviate free heme levels to rescue BACH1 protein levels back, and not the transcripts. The counteractions between BACH1 and NRF2 play a critical role for managing cellular stresses by turning on and off antioxidant gene expression. Therefore, extracellular or intracellular interruption of the BACH1 and NRF2 regulatory loop could be detrimental for cells burdening reactive oxygen species (ROS) stress or ROS-mediated signaling for tumorigenesis.

 doi:10.20944/preprints202101.0626.v1

Exogenous antioxidant treatment such as N-acetyl cysteine (NAC) or vitamin E in a water soluble form stabilizes BACH1 levels by reducing intracellular free heme, which further facilitates metastasis of KRas mutated lung cancer cells [11]. Pharmacological inhibition of HMOX1 using ZnPPIX was able to suppress lung cancer metastasis by inducing Bach1-destabilization in a Fbox22-dependent manner [12].

A consequential product of hyperactivated mitochondrial respiration, ROS, is increased in shBACH1 TNBC cells [10]. In particular, mitochondrial ROS was significantly higher in shBACH1 cells, while cytoplasmic ROS showed similar levels in shBACH1 cells compared to controls. Induced ROS levels by BACH1 depletion did not affect either cell survival or cell death, because BACH1 depletion induces expression of glutathione (GSH) biosynthesis such as glutamate-cysteine ligase modifier (*Gclm*) and catalytic subunit (*Gclc),* and cystine/glutamate antiporter xCT, encoded by solute carrier family 7 member 11 (*Slc7A11*) as a defense mechanism [49]. BACH1 depletion, as a defense mechanism, could de-repress expression of glutathione synthesis genes to counterbalance increased ROS.

Due to the fact that glutathione is known to inhibit ferroptosis which is a type of programmed cell death triggered by iron, BACH1 is also involved in the regulation of ferroptosis [49]. Combined analyses of ChIP-seq and RNA-sequencing using mouse myeloblast M1 cells identified Bach1-target genes including ferritin genes (*Fth1* and *Tfl1*) and ferroporin (encoded by solute carrier family 40 member 1, *Slc40a1*) by binding to the regulatory region. Further, expression of Bach1 target genes were validated using qPCR and western blotting in the Bach1$^{-/-}$ MEFs, indicating BACH1 involvement in lipoperoxidation and iron metabolism. Therefore, ferroptosis promoted by BACH1 could impair severity of diseases such as acute myocardial infarction [49].

8. BACH1 regulates glycolysis metabolism in cancer

Aerobic glycolysis of cancer cells in the presence of oxygen represents highly convergent metabolic phenotypes, providing a diagnostic foundation for cancer detection [1, 2, 4, 47]. There is evidence that BACH1 participates in regulation of glycolysis pathway in lung cancer cells [11]. Integrated analyses of RNA-seq and ChIP-seq to access BACH1 targets using lung adenocarcinoma uncovered that BACH1 activates transcription of hexokinase 2 (*HK2*) and Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) at their promoters as a transcriptional activator (**Figure 2**) [11]. Although *HK2* mRNA was the most significantly up-regulated gene in the cells after treatment with antioxidants, hexokinase 1 (*HK1*) was not changed. In addition, transcripts of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (*PFKFB3*) and solute-carrier family 16 member 1 (*SLC16A1*) was increased in cells treated with antioxidants, suggesting a positive correlation with BACH1 expression in patient tumors.

Antioxidant-treated cells showed higher glycolysis rates than control cells which are assessed by ECAR, OCR, higher glucose consumption, and higher lactate production in BACH1-dependent manner [11]. Furthermore, antioxidant-treated lung cancer cells showed increased metastasis through activated glycolysis. Higher ATP levels through glycolysis in the antioxidant treated cells became an energy source to support increased invasiveness and metastasis, in a Bach1-dependent manner. Activation of glycolysis pathway by exogenous expression of HK2 could induce invasiveness and metastasis of control lung cancer cells. Knockout of Bach1 using single guide RNA against Bach1 gene (sgBach1) normalized ATP levels, invasiveness, and metastasis of antioxidant-treated cancer cells to the levels of control. Therefore, inhibition of glycolysis pathways was effective to suppress cancer metastasis in both antioxidant treated and Bach1-induced lung cancer models. Small inhibitory molecules including 2-deoxyglucose (2-DG) and lonidamine for inhibition of HK2, 3-bromopyruvate (3-BP) for inhibition of GAPDH, dichloroacetate (DCA) for inhibition of PDK, and AZD3965 for inhibition of monocarboxylate transporter (MCT1, encoded by *SLC16A1*) resulted in reduced migration or metastasis of antioxidant-treated lung cancer cells. However, inhibition of mitochondrial pyruvate carrier (MPC)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 January 2021 doi:10.20944/preprints202101.0626.v1

using UK5099 or inhibition of pentose phosphate pathway (PPP) using 6-aminonicotinamide (6-AN) did not change migration of either antioxidant treated or control lung cancer cells. Hence, glycolysis inhibition could present BACH1-mediated metastatic process of lung cancer, as inhibition of BACH1 using either shRNA, sgRNA, or hemin treatment decreases migration and metastasis of cancer cells through aerobic glycolysis reduction.

9. Targeting BACH1 is clinically beneficial

The functional role of BACH1 for cancer metabolism was validated using the patient data cohorts such as the Cancer Genome Atlas (TCGA) including breast, lung adenocarcinoma, and pancreas ductal adenocarcinoma [10–12, 27]. It is clear that BACH1 serves as a diagnostic marker to stratify patient outcomes with numerous cancer types. Moreover, preclinical studies and bioinformatic approaches suggest that breast tumors expressing low levels of BACH1 might be potentially sensitive to metformin treatment, while those enriched with BACH1 might be resistant to metformin treatment when administered as a single agent to manage tumors. In other words, combining metformin with hemin (or any other possible BACH1 inhibitor) would effectively suppress growth of tumors from cancer patients who are potentially metformin resistant due to their enriched high BACH1 levels. The gene expression patterns in a gene signature consisting of BACH1 and mitochondrial ETC genes are clinically useful to predict outcomes of patients when administered with mitochondrial inhibitors such as metformin, which is currently in clinical trials.

In particular, blockade of BACH1 using either approach including shRNA, sgRNA, or small inhibitors such as hemin, reduced BACH1 levels in cancer cells and successfully and efficiently inhibited cancer metastasis processes (**Figure 3**). Also blockade of BACH1 rewires metabolic liability, which further generates metabolic vulnerability. Furthermore, inhibiting BACH1 downstream targets such as mitochondrial metabolic pathways, glycolysis pathways, heme regulatory pathways, and redox homeostasis might be a highly insightful approach to manage metastatic cancer in a complex and context-dependent tumor microenvironment.

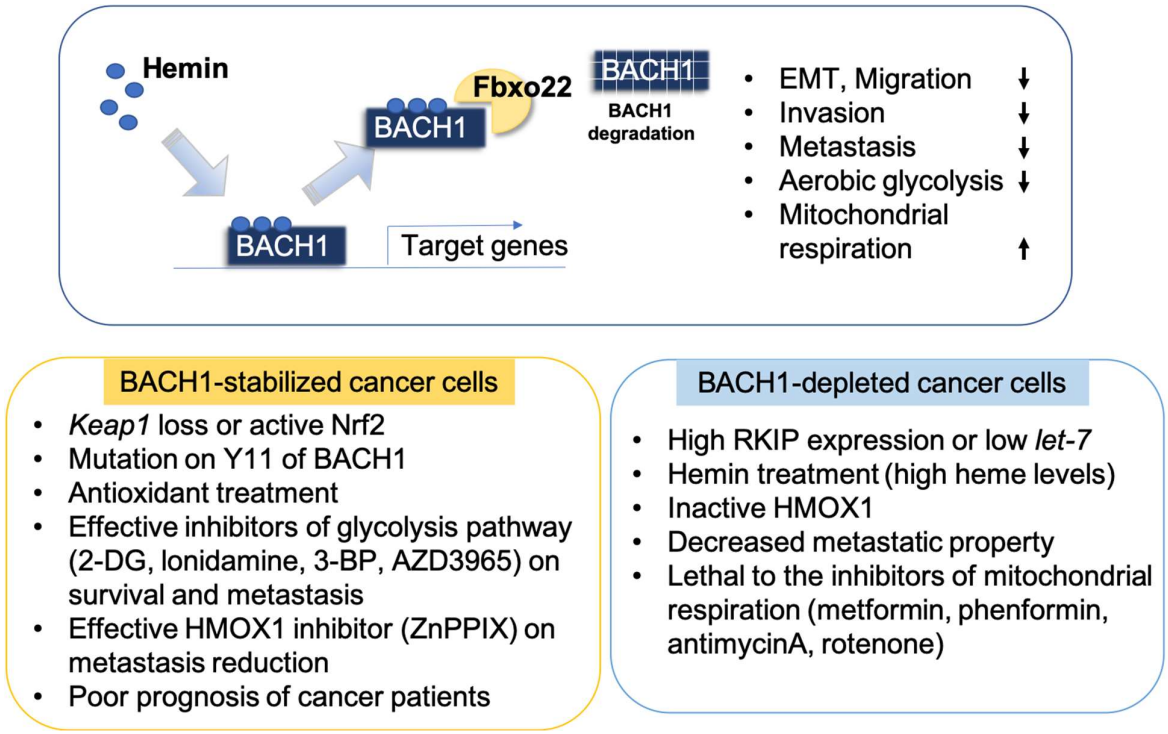

Figure 3. Pharmacological inhibition of BACH1 as cancer therapeutics. Hemin is a non-toxic BACH1 drug, causing degradation of BACH1 through ligase Fbxo22 interaction with BACH1. BACH1 depletion modifies transcriptional

expression of BACH1 target genes both positively and negatively, decreasing EMT, migration, invasiveness, metastasis of cells, and aerobic glycolysis, but increasing mitochondrial oxidative phosphorylation. Alteration of BACH1 levels and cellular phenotypic changes by BACH1 levels are summarized.

10. Conclusion

Cancer metabolism is a proven therapeutic target. Distinct cancer metabolism that supports rapid tumor growth became a crucial resource to achieve effective treatments of cancer as well as to identify accurate prognostics and cancer diagnostics in clinics. Currently, numerous drugs that inhibit metabolic pathways including nucleic acid biosynthesis, folate metabolism, and amino acid metabolism have been approved for cancer treatment and widely used for many cancers. Above all, plasticity of cancer metabolism generates ineffectiveness of a drug inhibiting metabolic pathways unless their metabolism is rewired to be targetable. Therefore, understanding cancer-specific metabolism and its molecular mechanism to restrict metabolic plasticity of cancer cells will provide an effective therapeutic strategy targeting key metabolisms.

In conclusion, BACH1 is a useful therapeutic target to achieve effective cancer treatment as highlighted by recent numerous publications. BACH1 regulates cancer metabolism processes such as glycolysis and mitochondrial OXPHOS, and redox regulation. Inhibition of BACH1 could be achieved by hemin in animal models, suggesting its broad application with low toxicity. Particularly, targeting BACH1 with a combination of metabolic inhibitors will be a promising therapeutic intervention. While recent studies identified new roles of BACH1 in cancer metabolism, there are still areas to understand such as other metabolic pathway regulation by BACH1 in tumors including: one carbon metabolism, amino acid, pentose phosphate pathway, and fatty acid oxidation. As briefly mentioned earlier, BACH1 plays a role for macrophage metabolism, which might be a key factor for communication between cancer and tumor-infiltrated macrophages. It is important to explore metabolic communication between cancer cells and immune cells infiltrated into tumors in future studies [50].

Author Contributions: For review article, "Conceptualization, J.L.; writing—original draft preparation. J.P and J.L.; writing—review and editing, J.P and J.L.; funding acquisition, J.L.

Funding: This work was supported by the Department of Defense Congress Directed medical research program, Breast Cancer Research Program Break Through Award Level I (BC161588 to JL).

Conflicts of Interest: The author declares no conflict of interest.

References

1. M. G. Vander Heiden and R. J. DeBerardinis, "Understanding the Intersections between Metabolism and Cancer Biology," *Cell*, vol. 168, no. 4, pp. 657–669, Feb. 2017, doi: 10.1016/j.cell.2016.12.039.
2. O. Warburg, "On the origin of cancer cells," *Science*, vol. 123, no. 3191, pp. 309–314, Feb. 1956, doi: 10.1126/science.123.3191.309.
3. R. A. Cairns and T. W. Mak, "The current state of cancer metabolism," *Nature Reviews Cancer*, Sep. 23, 2016. https://www.nature.com/articles/nrc.2016.100 (accessed Oct. 21, 2019).
4. R. J. DeBerardinis and N. S. Chandel, "Fundamentals of cancer metabolism," *Science Advances*, vol. 2, no. 5, p. e1600200, May 2016, doi: 10.1126/sciadv.1600200.
5. L. Bar-Peled and D. M. Sabatini, "Regulation of mTORC1 by amino acids," *Trends Cell Biol*, vol. 24, no. 7, pp. 400–406, Jul. 2014, doi: 10.1016/j.tcb.2014.03.003.
6. C. Commisso *et al.*, "Macropinocytosis of protein is an amino acid supply route in Ras-transformed cells," *Nature*, vol. 497, no. 7451, pp. 633–637, May 2013, doi: 10.1038/nature12138.
7. A. J. Wolpaw and C. V. Dang, "Exploiting Metabolic Vulnerabilities of Cancer with Precision and Accuracy," *Trends in Cell Biology*, vol. 28, no. 3, pp. 201–212, Mar. 2018, doi: 10.1016/j.tcb.2017.11.006.
8. J. Lee *et al.*, "Network of mutually repressive metastasis regulators can promote cell heterogeneity and metastatic transitions," *PNAS*, vol. 111, no. 3, pp. E364–E373, Jan. 2014, doi: 10.1073/pnas.1304840111.
9. S.-M. Fendt, C. Frezza, and A. Erez, "Targeting Metabolic Plasticity and Flexibility Dynamics for Cancer Therapy," *Cancer Discov*, vol. 10, no. 12, pp. 1797–1807, Dec. 2020, doi: 10.1158/2159-8290.CD-20-0844.

10. J. Lee *et al.*, "Effective breast cancer combination therapy targeting BACH1 and mitochondrial metabolism," *Nature*, vol. 568, no. 7751, pp. 254–258, Apr. 2019, doi: 10.1038/s41586-019-1005-x.
11. C. Wiel *et al.*, "BACH1 Stabilization by Antioxidants Stimulates Lung Cancer Metastasis," *Cell*, vol. 178, no. 2, pp. 330-345.e22, Jul. 2019, doi: 10.1016/j.cell.2019.06.005.
12. L. Lignitto *et al.*, "Nrf2 Activation Promotes Lung Cancer Metastasis by Inhibiting the Degradation of Bach1," *Cell*, vol. 178, no. 2, pp. 316-329.e18, Jul. 2019, doi: 10.1016/j.cell.2019.06.003.
13. K. Igarashi, T. Kurosaki, and R. Roychoudhuri, "BACH transcription factors in innate and adaptive immunity," *Nature Reviews Immunology*, vol. 17, no. 7, pp. 437–450, Jul. 2017, doi: 10.1038/nri.2017.26.
14. Y. Dohi *et al.*, "Bach1 inhibits oxidative stress–induced cellular senescence by impeding p53 function on chromatin," *Nature Structural & Molecular Biology*, vol. 15, no. 12, Art. no. 12, Dec. 2008, doi: 10.1038/nsmb.1516.
15. J. Li *et al.*, "Phosphorylation of BACH1 switches its function from transcription factor to mitotic chromosome regulator and promotes its interaction with HMMR," *Biochem. J.*, vol. 475, no. 5, pp. 981–1002, 15 2018, doi: 10.1042/BCJ20170520.
16. Mito Shinji *et al.*, "Myocardial Protection Against Pressure Overload in Mice Lacking Bach1, a Transcriptional Repressor of Heme Oxygenase-1," *Hypertension*, vol. 51, no. 6, pp. 1570–1577, Jun. 2008, doi: 10.1161/HYPERTENSIONAHA.107.102566.
17. T. Oyake *et al.*, "Bach proteins belong to a novel family of BTB-basic leucine zipper transcription factors that interact with MafK and regulate transcription through the NF-E2 site.," *Mol Cell Biol*, vol. 16, no. 11, pp. 6083–6095, Nov. 1996.
18. X. Zhang *et al.*, "Bach1: Function, Regulation, and Involvement in Disease," *Oxid Med Cell Longev*, vol. 2018, p. 1347969, 2018, doi: 10.1155/2018/1347969.
19. G. D. Amoutzias *et al.*, "One billion years of bZIP transcription factor evolution: conservation and change in dimerization and DNA-binding site specificity," *Mol Biol Evol*, vol. 24, no. 3, pp. 827–835, Mar. 2007, doi: 10.1093/molbev/msl211.
20. A. W. Reinke, J. Baek, O. Ashenberg, and A. E. Keating, "Networks of bZIP Protein-Protein Interactions Diversified Over a Billion Years of Evolution," *Science*, vol. 340, no. 6133, pp. 730–734, May 2013, doi: 10.1126/science.1233465.
21. K. Ogawa *et al.*, "Heme mediates derepression of Maf recognition element through direct binding to transcription repressor Bach1," *EMBO J.*, vol. 20, no. 11, pp. 2835–2843, Jun. 2001, doi: 10.1093/emboj/20.11.2835.
22. G. P. Moss, P. a. S. Smith, and D. Tavernier, "Glossary of class names of organic compounds and reactivity intermediates based on structure (IUPAC Recommendations 1995)," *Pure and Applied Chemistry*, vol. 67, no. 8–9, pp. 1307–1375, Jan. 1995, doi: 10.1351/pac199567081307.
23. J. Sun *et al.*, "Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene," *EMBO J*, vol. 21, no. 19, pp. 5216–5224, Oct. 2002, doi: 10.1093/emboj/cdf516.
24. Y. Zenke-Kawasaki *et al.*, "Heme Induces Ubiquitination and Degradation of the Transcription Factor Bach1," *Molecular and Cellular Biology*, vol. 27, no. 19, pp. 6962–6971, Oct. 2007, doi: 10.1128/MCB.02415-06.
25. H.-J. Warnatz *et al.*, "The BTB and CNC Homology 1 (BACH1) Target Genes Are Involved in the Oxidative Stress Response and in Control of the Cell Cycle," *J Biol Chem*, vol. 286, no. 26, pp. 23521–23532, Jul. 2011, doi: 10.1074/jbc.M111.220178.
26. M. Matsumoto *et al.*, "Genomewide approaches for BACH1 target genes in mouse embryonic fibroblasts showed BACH1-Pparg pathway in adipogenesis," *Genes to Cells*, vol. 21, no. 6, pp. 553–567, 2016, doi: 10.1111/gtc.12365.
27. U. Lee *et al.*, "A prognostic gene signature for metastasis-free survival of triple negative breast cancer patients," *PLoS ONE*, vol. 8, no. 12, p. e82125, 2013, doi: 10.1371/journal.pone.0082125.
28. J. Yun *et al.*, "Signalling pathway for RKIP and Let-7 regulates and predicts metastatic breast cancer," *EMBO J.*, vol. 30, no. 21, pp. 4500–4514, Aug. 2011, doi: 10.1038/emboj.2011.312.
29. Y. Liang *et al.*, "Transcriptional Network Analysis Identifies BACH1 as A Master Regulator of Breast Cancer Bone Metastasis," *J. Biol. Chem.*, p. jbc.M112.392332, Aug. 2012, doi: 10.1074/jbc.M112.392332.
30. M. Sato *et al.*, "BACH1 Promotes Pancreatic Cancer Metastasis by Repressing Epithelial Genes and Enhancing Epithelial–Mesenchymal Transition," *Cancer Res*, vol. 80, no. 6, pp. 1279–1292, Mar. 2020, doi: 10.1158/0008-5472.CAN-18-4099.
31. M. Fang, J. Ou, L. Hutchinson, and M. R. Green, "The BRAF Oncoprotein Functions through the Transcriptional Repressor MAFG to Mediate the CpG Island Methylator Phenotype," *Molecular Cell*, vol. 55, no. 6, pp. 904–915, Sep. 2014, doi: 10.1016/j.molcel.2014.08.010.
32. M. Fang, L. Hutchinson, A. Deng, and M. R. Green, "Common BRAF(V600E)-directed pathway mediates widespread epigenetic silencing in colorectal cancer and melanoma," *PNAS*, vol. 113, no. 5, pp. 1250–1255, Feb. 2016, doi: 10.1073/pnas.1525619113.
33. S. Dangi-Garimella *et al.*, "Raf kinase inhibitory protein suppresses a metastasis signalling cascade involving LIN28 and let-7," *EMBO J*, vol. 28, no. 4, pp. 347–358, Feb. 2009, doi: 10.1038/emboj.2008.294.
34. J. Sun *et al.*, "The Promoter of Mouse Transcription Repressor bachl Is Regulated by Spl and Trans-Activated by Bachl," *J Biochem*, vol. 130, no. 3, pp. 385–392, Sep. 2001, doi: 10.1093/oxfordjournals.jbchem.a002997.
35. D. P. Bartel, "Metazoan MicroRNAs," *Cell*, vol. 173, no. 1, pp. 20–51, Mar. 2018, doi: 10.1016/j.cell.2018.03.006.
36. S. Li, T. Chen, Z. Zhong, Y. Wang, Y. Li, and X. Zhao, "microRNA-155 silencing inhibits proliferation and migration and induces apoptosis by upregulating BACH1 in renal cancer cells," *Molecular Medicine Reports*, vol. 5, no. 4, pp. 949–954, Apr. 2012, doi: 10.3892/mmr.2012.779.

37. S. Shirjang *et al.*, "miR-330 Regulates Colorectal Cancer Oncogenesis by Targeting BACH1," *Adv Pharm Bull*, vol. 10, no. 3, pp. 444–451, Jul. 2020, doi: 10.34172/apb.2020.054.
38. S. C. Slater *et al.*, "MicroRNA-532-5p Regulates Pericyte Function by Targeting the Transcription Regulator BACH1 and Angiopoietin-1," *Molecular Therapy*, vol. 26, no. 12, Art. no. 12, 2018, doi: 10.1016/j.ymthe.2018.08.020.
39. H. Go *et al.*, "MiR-196a regulates heme oxygenase-1 by silencing Bach1 in the neonatal mouse lung," *American Journal of Physiology-Lung Cellular and Molecular Physiology*, vol. 311, no. 2, pp. L400–L411, Jun. 2016, doi: 10.1152/ajplung.00428.2015.
40. N. Tanioka *et al.*, "Induction of hepatic Bach1 mRNA expression by carbon tetrachloride-induced acute liver injury in rats," *Biomedical Reports*, vol. 2, no. 3, pp. 359–363, May 2014, doi: 10.3892/br.2014.235.
41. D. M. Bissell, K. E. Anderson, and H. L. Bonkovsky, "Porphyria," *N. Engl. J. Med.*, vol. 377, no. 9, pp. 862–872, Aug. 2017, doi: 10.1056/NEJMra1608634.
42. D. Chiabrando, F. Vinchi, V. Fiorito, S. Mercurio, and E. Tolosano, "Heme in pathophysiology: a matter of scavenging, metabolism and trafficking across cell membranes," *Front. Pharmacol.*, vol. 5, 2014, doi: 10.3389/fphar.2014.00061.
43. H. Suzuki, S. Tashiro, J. Sun, H. Doi, S. Satomi, and K. Igarashi, "Cadmium Induces Nuclear Export of Bach1, a Transcriptional Repressor of Heme Oxygenase-1 Gene," *J. Biol. Chem.*, vol. 278, no. 49, pp. 49246–49253, Dec. 2003, doi: 10.1074/jbc.M306764200.
44. W. Hou, Y. Shan, J. Zheng, R. W. Lambrecht, S. E. Donohue, and H. L. Bonkovsky, "Zinc Mesoporphyrin Induces Rapid and Marked Degradation of the Transcription Factor Bach1 and Up-regulates HO-1," *Biochim Biophys Acta*, vol. 1779, no. 3, pp. 195–203, Mar. 2008, doi: 10.1016/j.bbagrm.2008.01.006.
45. O. C. Attucks *et al.*, "Induction of Heme Oxygenase I (HMOX1) by HPP-4382: A Novel Modulator of Bach1 Activity," *PLOS ONE*, vol. 9, no. 7, p. e101044, Jul. 2014, doi: 10.1371/journal.pone.0101044.
46. K. Vasan, M. Werner, and N. S. Chandel, "Mitochondrial Metabolism as a Target for Cancer Therapy," *Cell Metabolism*, vol. 32, no. 3, pp. 341–352, Sep. 2020, doi: 10.1016/j.cmet.2020.06.019.
47. P. Lee, N. S. Chandel, and M. C. Simon, "Cellular adaptation to hypoxia through hypoxia inducible factors and beyond," *Nat Rev Mol Cell Biol*, vol. 21, no. 5, pp. 268–283, May 2020, doi: 10.1038/s41580-020-0227-y.
48. D. Y. Gui *et al.*, "Environment Dictates Dependence on Mitochondrial Complex I for NAD+ and Aspartate Production and Determines Cancer Cell Sensitivity to Metformin," *Cell Metab.*, vol. 24, no. 5, pp. 716–727, 08 2016, doi: 10.1016/j.cmet.2016.09.006.
49. H. Nishizawa *et al.*, "Ferroptosis is controlled by the coordinated transcriptional regulation of glutathione and labile iron metabolism by the transcription factor BACH1," *J. Biol. Chem.*, vol. 295, no. 1, pp. 69–82, Jan. 2020, doi: 10.1074/jbc.RA119.009548.
50. C. A. Lyssiotis and A. C. Kimmelman, "Metabolic Interactions in the Tumor Microenvironment," *Trends Cell Biol.*, vol. 27, no. 11, pp. 863–875, 2017, doi: 10.1016/j.tcb.2017.06.003.