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Complete List of Authors:	Eguchi, Takanori; Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Calderwood, Stuart; harvard, Takigawa, Masaharu; Okayama University Graduate School of Medicine and Dentistry, Biochemistry and Molecular Dentistry Kubota, Satoshi; Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Derpartment of Biochemistry and Molecular Dentistry
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Intracellular MMP3 Promotes *HSP* Gene Expression in Collaboration with Chromobox Proteins

Takanori Eguchi[1,2,3,4,*], Stuart K. Calderwood[1,*] Masaharu Takigawa[3], Satoshi Kubota[5], Ken-ichi Kozaki[2]

[1]Department of Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115, USA

[2]Department of Dental Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, 700-8525, Japan

[3]Advanced Research Center for Oral and Craniofacial Sciences, Okayama University Dental School / Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, 700-8525, Japan

[4]Department of Oral Disease Research, Research Institute, National Center for Geriatrics and Gerontology, Obu, Aichi 474-8511, Japan

[5]Department of Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, 700-8525, Japan

*Correspondence should be addressed to:

Takanori Eguchi, DDS, PhD
2-5-1, Shikata-cho, Kita-ku, Okayama city, 700-8525, Japan
Phone: +81-86-235-6662
Fax: +81-86-235-6664
E-mail: eguchi@okayama-u.ac.jp

Stuart K. Calderwood, PhD
3 Blackfan Circle CLS-610, Boston, MA 02115, USA
Phone: +1-617-735-2947
Fax: +1-617-735-2814
E-mail: scalderw@bidmc.harvard.edu

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E-mail addresses

Takanori Eguchi[1,2,3,*] eguchi@okayama-u.ac.jp

Satoshi Kubota[4] kubota1@md.okayama-u.ac.jp

Masaharu Takigawa[5] takigawa@md.okayama-u.ac.jp

Ken-ichi Kozaki[2] ken-1@okayama-u.ac.jp

Stuart K. Calderwood[1,*] scalderw@bidmc.harvard.edu

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Abstract (216 words)

Matrix metalloproteinases (MMPs) are crucial factors in tumor progression, inflammatory/ immune responses and tissue development / regeneration. Of note, it has been known that MMPs promote genome instability, epithelial-mesenchymal transition, invasion and metastasis in tumor progression. We previously reported that human MMP3 could translocate into cellular nuclei and control transcription in human chondrosarcoma-derived cells and in articular cartilage (Mol Cell Biol, 2008, 28(7), 2391-2413); however, further transcriptional target genes and cofactors of intranuclear MMP3 have not been uncovered. In this paper, we used transcriptomics analysis in order to examine novel transcriptional target genes regulated by intracellular MMP3. We found that mRNA levels of *HSP* family members (*HSP70B', HSP72, HSP40/DNAJ* and *HSP20/CRYAB*) are upregulated by the intracellular MMP3 overload. Bioinformatic analysis predicted several transcription factors that possibly interact with MMP3. Among these factors, heat shock factor 1(HSF1) cooperated with the MMP3 to activate the *HSP70B'* gene promoter in reporter gene assays, while a dominant negative HSF1 blocked the role for MMP3 in the *trans*-activation. The hemopexin-like repeat (PEX) domain of the human MMP3 was essential for transcriptional induction of the *HSP70B'* gene. In addition, chromobox proteins CBX5/HP1α and CBX3/HP1γ cooperated with the PEX domain in induction of *HSP70B'* mRNA. Taken together, this study newly clarified that intracellular MMP3 cooperate with CBXs/HP1s in transcriptional promotion of *HSP* genes.

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INTRODUCTION

MMP family members are critical in tumor progression [1-4], in other inflammatory diseases [5] and in physiological tissue remodeling, wound healing and development [6-8]. Of note, it has been known that MMPs promote invasion and metastasis in tumor progression [1-4]. MMPs were found initially to degrade extracellular matrix (ECM) proteins, such as some types of collagens and proteoglycans [9]. Such protelytic activity of MMPs is required for physiological tissue turnover and remodeling, and for tumor cell migration, invasion, and metastasis. Later, MMPs have been shown to proteolytically process growth factors, cytokines, and their receptors, leading to altered activities of these molecules [10-12]. In addition, it was reported that MMP3 can induce genome instability and epithelial-to-mesenchymal transition [13]. However, MMPs may play important roles inside cells as well. We have reported that intracellular MMP3 controls transcription of the *CCN2/CTGF* gene in HCS-2/8, a human chondrosarcoma cell line, and in MDA-MB-231 breast carcinoma cells [14,15]. More recently, it was reported that intracellular MMP12 could control transcription of the *NFKBIA* gene encoding IkBα during anti-viral immunity [16]. Separately from such roles for MMPs in transcriptional regulation, roles for intracellular MMPs in cell death have been reported as well. Kwan et al. also showed that MMP2 is present in the nuclei of cardiac myocytes and can induce apoptosis [17]. Si-Tayeb et al. reported that MMP3 is present in the cell nuclei and induces apoptosis [18]. Thus, MMPs appear to play intracellular roles in diverse diseases. However, the key transcriptional targets controlled by the intracellular MMP3 have not yet been unveiled. Nuclear MMP3-associated proteins (NuMAP) was identified previously and a member of chromobox protein CBX3/HP1γ was shown to cooperate with MMP3 in transcriptional regulation [14,15]. In the present study, we show that another chromobox protein CBX5/HP1α also cooperate with MMP3 in transcriptional regulation.

In the present study, we examined transcriptomic screening in order to find downstream factors that are regulated by intracellular MMP3. We found some members of heat shock protein (HSP) gene family are induced by MMP3. HSP family members have been known to bind

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loosely to proteins and promote protein folding or induce proteasomal or lysosomal degradation of proteins [19]. *de novo* translated proteins or structurally unfolded proteins under various cell signals are loosely bound by HSPs that fold or refold proteins that acquire physiological functions or are degraded in proteasomes or lysosomes [19]. HSP insufficiency is thought to cause accumulation of unfolded proteins and protein aggregation that are a cause of diseases such as neurodegeneration. It has been shown that cancer cells possess elevated HSP levels that is mediated by heat shock transcription factor (HSF1) [20-23]. Activated HSF1 has been shown to be associated with poor prognosis in cancers [24-26]. For example, nutrient such as amino acids and growth factor signals such as IGF signal are received by mTOR that promote translational protein synthesis leading to upregulation of cellular metabolism and survival; mTOR also receive stress signals and then activate / phosphorylates HSF1 at serine 326 leading to activation of HSP genes [27]. In addition, in a model of spontaneous mammary tumor progression and metastasis with *hsp70* gene knockout, we recently showed that Hsp70 activated c-Met oncogene that is a key factor in cancer invasion and metastasis [28]. Therefore, the MMP3-HSP conduction is thought to be a novel tumor progressing signal.

MATERIALS AND METHODS

Plasmid constructs

pFlag3-MMP3-myc, pFlag3-PEX-myc, pcDNA3.1/HP1g/CBX3-HA3 were described previously [14]. For the construction of MMP3-GFP expression plasmid, cDNA of MMP3 including signal peptide-coding region was amplified by PCR for DNA topoisomerase-mediated subcloning into the pcDNA3.1/CT-GFP-TOPO vector (Invitrogen, Carlsbad, CA). Gateway entry cDNA clone of HP1α, CNOT4 and MLL5 were provided from NITE NBRC and subcloned into pcDNA/V5-His TOPO vector (Invitrogen) with C-terminal V5-His tags, and designated pcDNA3.1/HP1A-V5His, pcDNA3.1/CNOT4-V5His, pcDNA3.1/MLL5-V5His, respectively. pcDNA/His/DN-HSF1.C383 was cloned by generating stop codon using a quick-change method from pcDNA/His/HSF1 [32].

Cells, heat shock

HEK293, HeLa and COS-7 were cultured in DMEM containing 10% FBS. Plasmid DNA transfection was carried out as described previously [14]. For heat shock, the medium was replaced to incubated medium and then put in a water bath.

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Real-time qRT-PCR

Real-time qRT-PCR was carried out as described previously [14]. Total RNA was prepared using miRNeasy RNA purification system (Qiagen, Germany) with DNase I treatment. cDNA was synthesized using RT kit for qRT-PCR (Qiagen, Germany) with a mixture of oligo dT and random primers after genomic DNA elimination. cDNA pool was diluted 5 to 20 fold according to target genes. cDNA standard with permissible slopes of PCR efficiencies was prepared from step dilution of the cDNA pool for relative quantification of mRNA levels. In a 20 ul of the qPCR mix, 0.25 □M of each primer, 4-10 ul of diluted cDNA sample, and 10 ul SYBR green 2x Master Mix (Applied biosystems) were reacted. Dissociation curves with specific single-peaked PCR and proper amplification slopes for PCR efficiency were confirmed.

Microarray/Gene chip analyses

HEK293 cells were cultured in the 6-well plate. Plasmid DNA was transfected as described previously [14]. Total RNA was prepared at 18 h after the transfection. cDNA was synthesized as described above. Human Gene 1.0 ST Array (Affymetrix) was used for transcriptome analysis. Gene spring was used for data mining.

KeyMolnet, pathway analysis and gene ontology

Data obtained in microarray were input to KeyMolnet [33]. The cDNA levels altered more than 1.5-fold between the experimental conditions were used for analyses. Key molecular networks of transcription factor cascades and cell signaling pathways were searched and scored. Pathological events and diseases related to the networks were searched and scored.

Luciferase assay

Luciferase assay was carried out as described [14]. Cells were cultured in 96-well plates and transfected at 50 to 70 % confluency with 25 ng of reporter and 100 ng of effector plasmid using 0.4 ul of FuGENE HD (Roche) per well. Cells were stimulated with heat shock at 16-20 hrs after the transfection. At 40-48 hrs after the transfection, 70 ul medium was removed, and 30 ul Bright-Glo reagent (Promega) was added to 30 ul of remaining medium. Cells were further lysed by pipetting several times, incubated for 5 min at 37℃and pipetted again. The lysate (40 ul) was transferred to 96-wells white plate and luminescence was measured in a microplate luminometer (Turner design). Relative light units (RLU) were calculated from measured luciferase activities (n=4).

Gene sequences, primers

TaKaRa perfect real-time PCR system and Primer 3 provide by MIT were used for the design of primers. Information of gene sequences was obtained from Ref-seq, NCBI. For qRT-PCR, all the primer pairs were designed at independent exons or exon-exon junctions. *HSPA6 /*

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HSP70B'/BC035665, 5'-ATG AAG CCG AGC AGT ACA AG-3' and 5'-TTG TCC CTA AGG CTT TCC-3'; Sequences of primers for human MMP3, CTGF/CCN2, GAPDH were described previously [14].

RESULTS

Intracellular MMP3 Up-Regulated mRNA Levels of *MMP* and *HSP Genes*.

Our previous work suggested a role for MMP3 in transcription [14,15]. In this paper, we examined the genome-wide effects of intracellular MMP3 overexpression on the transcriptome. We carried out microarray/gene-chip analysis with pFlag3-MMP3-myc-transfected HEK293 cells and with vector controls (Fig. 1A). We have listed the top 10 upregulated and top 10 downregulated genes at the mRNA level in intracellular MMP3-overexpressed cells compared with the control (Fig. 1B and C). As evidence that this experiment was successful, we found the mRNA level of the *MMP3* itself was higher in the MMP3-overexpressed cells than in the control. *MMP1* and *MMP10* were ranked in the top 10 increased mRNAs. Of highest significance, the *HSP70B'/HSPA6* mRNA level was 12.6-fold higher in the pFlag3-MMP3-myc-transfected cells. In addition, the *HSP72/HSPA1A/HSPA1B*, *HSP40/HSPDNAJ1/HDJ1,* and *HSP20/HSPB5/CRYAB* (encoding αB-crystallin) genes were ranked in the top 10 increased. Therefore, it was suggested that MMPs and HSPs may be transcriptionally controlled by the intracellular MMP3.

Based on the gene expression signature altered by the MMP3 overload, we carried out gene ontology analysis in order to specify biological and pathological events and diseases involving the intracellular MMP3. UV response, pluripotency, cancer, viral infection, metastasis and tight junction were highly scored as biological and pathological events involving MMP3 overexpression (Table 1). We predicted diseases involving the intracellular MMP3 overload based on the gene expression signature as well (Table 2). Rheumatoid arthritis (RA), Mantle cell lymphoma, colorectal cancer, lung cancer, prostate cancer and breast cancer were suggested

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as such MMP3-associated diseases.

Transcriptional Factors and Signaling Pathways Associated with Intracellular MMP3.

We searched MMP3-related transcription factors that can directly control transcription of such *MMP, HSP* and other MMP3-altered genes with KeyMolnet analysis (Fig. 2). In the regulatory map, the MMP3-related transcription factors were shown in blue, while, and purple. The target genes were shown in red. In addition, mediators of diseases, targets of drugs, factors involved in the pathological events and biomarkers were predicted in this bioinformatics. Interestingly, the stress-responsive, oncogenic HSF1, c-Myc oncoprotein, p53 tumor suppressor, oxidative stress responsive Nrf2, and the other factors including FOXO3, VDR, Ets-1, CUTL1, TBP, SP1 were listed as the intracellular MMP3-related transcription factors. Further upstream factors hnRNPK and NONO may regulate c-Myc. These factors shown in the map might be functionally altered by the intracellular MMP3. Action of MMP3 on the oncogenic stress-related p53, NRF2, c-myc, HSF1 and FoxO3 was implicated. We listed the MMP3-related signaling pathways as well using the KeyMolnet algorithm (Table 3). These signaling pathways may be involved in the role for MMP3 in transcriptional control of a range of processes.

Intracellular MMP3 and HSF1 Cooperatively Control a Promoter Region of *HSP70B'/HSPA6* Gene

As mRNA levels of *HSP* genes were up-regulated by the intracellular MMP3 overload, we next examined co-regulatory effects of MMP3 and HSF1. We co-transfected the plasmids expressing HSF1 or a dominant negative form of HSF1 (DN-HSF1) with pFlag3-MMP3-myc and the reporter constructs (Fig. 3A). As a control study, we confirmed that the *HSP70B'* promoter was activated by heat shock stress at 43℃for 30 min (Fig. 3B). Intracellular MMP3 overexpression also activated the *HSP70B'* promoter. Moreover, the overexpression of MMP3 and heat shock stress cooperatively activated the *HSP70B'* promoter.

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Next, we examined if HSF1 can be cooperative with the MMP3 overexpression in the *HSP70B'* promoter regulation. The activities from the *HSP70B'* promoter were increased by the overexpression of either HSF1 or MMP3, and additively increased by their co-overexpression (Fig. 3C). DN-HSF1 overexpression did not activate the *HSP70B'* promoter and did block the effect of MMP3 on the *HSP70B'* promoter. These results indicate that the MMP3 positively regulates *HSP70B'* gene in collaboration with HSF1.

The PEX Domain is Essential for Transcriptional Promotion of *HSP70B'* gene

The PEX domain of MMPs have been known as intermolecular interaction domain. Therefore, we hypothesized that the PEX domain could mediate MMP3-induced *HSP70B'* gene expression (Fig. 4A). As a pilot study before the microarray analysis, we confirmed that MMP3 mRNA was expressed in the pFlag3-MMP3-myc-transfected HEK293 cells (Fig. 4B). We further confirmed that *HSP70B'* mRNA was elevated by the MMP3 overexpression (Fig. 4C). We next asked whether the PEX domain of MMP3 could control *HSP70B'* gene expression. Interestingly, PEX domain overexpression up-regulated *HSP70B'* mRNA in HEK293 cells (Fig. 4D). Moreover, the PEX domain elevated activity of the *HSP70B'* promoter in HEK293 cells (Fig. 4E), while the promoterless negative control reporter did not respond to the PEX overexpression. These results indicate that the PEX domain is sufficient for the activation of *HSP70B'* promoter and subsequent induction of *HSP70B'* mRNA.

The PEX Domain and HP1 Cooperatively Induce *HSP70B'* Gene Expression

In a previous study, we identified nuclear MMP3-associated proteins (termed as NuMAPs) [14]. HP1α /CBX5, HP1γ /CBX3, KMT2E/MLL5 and CNOT4 are a part of the protein component of NuMAPs (Fig. 5A). We next examined potential cooperative roles for these factors with the PEX domain of MMP3 in *HSP70B'* gene regulation. CBX5/HP1α,

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CBX3/HP1γ, KMT2E/MLL5 or CNOT4 overexpression barely altered mRNA expression levels of *HSP70B'* while CBX5/HP1α and CBX3/HP1γ in combination with PEX domain overexpression elevated *HSP70B'* mRNA levels (Fig. 5B). KMT2E/MLL5 acted in a repressive manner while CNOT4 slightly up-regulated PEX-induced *HSP70B'* mRNA levels. Thus, these data indicate that chromobox proteins CBX5 and CBX3 are crucial cofactors under the interaction with the PEX domain.

MMP3-GFP Translocated into Cellular Nuclei and was Associated with Chromatin.

MMP3 has been known largely as a secretory extracellular protease. However, we found that human MMP3 localized in cellular nuclei and bound to DNA [14]. Indeed, six nuclear localization signals containing basic amino acid clusters have been found in the human MMP3 [14]. Interestingly, extracellular recombinant MMP can be translocated into nuclei [14]. Human MMP3 is synthesized with N-terminal signal peptide that is composed of 17 amino acids, although this signal peptide contains a proline residue at the center, which can reduce the functionality of the signal peptide. Next, to further confirm the behavior of MMP3 as a transcription factor, we therefore examined subnuclear localization of MMP3-GFP fusion protein by overexpressing it in COS7 cells (Fig. 6A). We prepared nuclear extracts from these cells that overexpressed MMP3-GFP. Thereafter, the nuclear extract was separated to soluble nuclear fraction, benzonase-treated chromatin fraction (Benzo.) and benzonase-resistant fraction (Resist.) (Fig. 6B).

Interestingly, MMP3-GFP was detected mostly in the benzonase-soluble chromatin fraction (lane 4) and in the soluble nuclear fractions (lane 2). This result indicated that MMP3-GFP localized in nuclei and bound to DNA / chromatin, even though expressed with the signal peptide. The chromobox protein CBX5/HP1α was mostly localized in soluble nuclear and chromatin fraction (lanes 1-4). Histone H3 was detected in the all fractions prepared, but especially in the benzonase-soluble and resistant chromatin fractions (lanes 3-6). These data

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indicated that MMP3, even is possession of a signal peptide can enter the nucleus and interact with CBX5/HP1α and histone H3 on chromosomes.

DISCUSSION

MMP3 is one of the major members of the MMP family and has crucial roles in tissue development, inflammation, cancer and other diseases. At the beginning of this study, without focusing on particular diseases or pathology, we bioinformatically analyzed signaling pathways, biological and pathological events. As a result of this approach, cancer and rheumatoid arthritis were predicted to be in the higher rank of processes regulated by MMP3, which was consistent with these predictions. A new finding that intracellular MMP3 is involved in cell stress regulation by the HSF1-HSP axis was uncovered, which could mediate MMP3-driven progression of tumor and arthritis. In addition to such search for upstream factors and pathway analysis, our experiment pursuing inter-molecular interaction of MMP3 revealed chromobox proteins / HP1s as essential cofactors of MMP3, whose cooperation with MMP3 in transcription was robustly demonstrated.

CBXs/HP1s was firstly shown as a heterochromatin forming protein that recognized methylated lysine residue at the 9th position of histone H3 and formed oligomers on such chromatin regions [29-33]. Muchardt et al. showed that coordinated methylated lysine and RNA binding is required for heterochromatin localization of mammalian HP1α [34]. Daujat et. al. showed that HP1 binds specifically to Lys26-methylated linker histone H1. 4 [35]. Kwon et al. showed that HP1 connects the FACT (Facilitation of Chromatin Transcription) histone chaperone complex to the phosphorylated CTD of RNA polymerase II [36]. Thus, HP1 can bind to methylated lysine and RNA. Considering these past findings together, we may conclude that HP1 is involved in MMP3-driven HSP gene activation (Fig. 7).

In the MMP3 molecule, the PEX domain has been shown as an intermolecular interaction domain lacking protease activity. Protease activity of MMP3 was dispensable for

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transcriptional control of *HSP70B'* gene; because the PEX domain alone robustly activated this gene. These findings suggest that the intracellular MMP3 can control transcription in a proteolytically non-activated condition; indeed, even in the absence of the protease domain. In addition, it is supposed that proteolytic activation of the intracellular MMP3 can trigger diverse biological changes including stress responses, pluripotency, tumorigenesis and arthritis. Molecular transport system of the intracellular MMP3 and further functions of MMP3 through interaction with cofactors in diseases are still under investigation. It should be noted that our data may be closely related to the development of cancer, in which expression of molecular chaperones, including Hsp70 is integral. Intracellular MMP3 may thus contribute to this process by increasing transcription of *HSP* genes in MMP3-expressing cancer cells.

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The authors declare no competing financial interests.

AUTHOR CONTRIBUTION STATEMENT

T.E. designed the study. T.E., M.T. and S.K.C. prepared materials. T.E. performed experiments. T.E. and S.K.C. analyzed data. S.K.C., S.K. and T.E. discussed. T.E., S.K.C., M.T., S.K. and K.K. wrote the manuscript.

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Tables

Table 1. Biological and pathological events involving intracellular MMP3 overexpression. The biological and pathological events were predicted from the gene expression signature under intracellular MMP3 overexpression.

Event	Score
UV response	31.006
Pluripotency	24.302
Cancer	23.999
Viral infection	16.134
Metastasis	16.003
Tight junction	15.746

Table 2. Diseases involving intracellular MMP3 overexpression. The diseases were predicted from the gene expression signature under intracellular MMP3 overexpression.

Disease	Score
Rheumatoid arthritis (RA)	23.77
Mantle cell lymphoma	16.809
Colorectal cancer	15.912
Lung cancer	13.921
Prostate cancer	13.836
Breast cancer	12.698

Table 3. Pathways and regulations involving intracellular MMP3 overexpression. The pathways and regulations were predicted from the gene expression signature under intracellular MMP3 overexpression.

Pathway and regulation	Score
MMP signaling pathway	16.566
Histone methylation	11.723
Transcriptional regulation by IRF	11.101
Transcriptional regulation by MRF	10.817
TG2 signaling pathway	10.188
Arginine methylation	8.974
HAT signaling pathway	8.159
ERK signaling pathway	7.951
Transcriptional regulation by Myc	7.366
IL-33 signaling pathway	7.231

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FIGURE LEGENDS

Figure 1. Gene expression signature upon intracellular MMP3 overexpression. (A) Scatter plot analysis of microarray. pFlag3-MMP3-myc or the empty vector was transfected into HEK293 cells. The mRNA levels were examined in microarray analysis. Red, high-level expression; Yellow, medium level; Blue, low-level. (B) Top 10 genes increased at the mRNA levels in the MMP3 overexpression. (C) Top 10 genes decreased at the mRNA levels in the MMP3 overexpression.

Figure 2. Transcriptional network involving intracellular MMP3. MMP3-regulated genes are shown in red. Common upstream factors are shown in blue. Second upstream factors are shown in purple. c-Myc can mediate signaling pathways between hnRNPK or NONO and the target HSP70. The numbers indicate #1, mediators of diseases. #2, targets of drugs. #3, factors involved in pathological events. #4, biomarkers. Genes altered at their expression levels by overexpression of MMP3 were shown in red.

Figure 3. Cooperative roles of intracellular MMP3 and HSF1 in regulation of *HSP70B'* promoter. (A) Domain structures of overexpressed MMP3, HSF1 and DN-HSF1. PG, peptide glycan binding domain also known as pre/pro-domains. PEX, hemopexin-like repeats. DBD, DNA binding domain; LZ, leucine zipper motif; RD, regulatory domain; AD, activation domain. (B) *HSP70B'* promoter-reporter response to intracellular MMP3 and heat shock stress. NH, non-heat. HS, heat shock at 43℃ for 30 min. (C) *HSP70B'* promoter response to intracellular MMP3 and HSF1. Luciferase activities of pGL2-*HSP70B'* or pGL2 basic. The reporter constructs were co-transfected with pFlag3-MMP3-myc, pcDNA3/His/HSF1, pcDNA3/His/DN-HSF1.C383 or the control vectors into HeLa cells.

Figure 4. Roles for intracellular MMP3 and PEX domain on *HSP70B'* gene expression. (A) Schematic structures of overexpressed MMP3 and PEX domain. (B) MMP3 mRNA expression upon intracellular MMP3 overexpression. pFlag3-MMP3-myc (MMP3) or the control vector (vec) was transfected into HEK293 cells. Total RNA was prepared after 30 h of the transfection. Relative mRNA levels of MMP3 to GAPDH are shown. (C) *HSP70B'* mRNA expression upon intracellular MMP3 overexpression. (D) *HSP70B'* mRNA expression upon intracellular PEX domain overexpression. (E) Luciferase activities from pGL2-basic vector or pGL2-*HSP70B'*. pFlag3-PEX-myc was co-transfected with the reporter constructs in HEK293 cells.

Figure 5. Cooperative roles for MMP3 PEX domain and HP1 in the induction of *HSP70B'* mRNA. (A) Structures of the PEX domain of MMP3 and cofactors KMT2E/MLL5, CBX5/HP1α, CBX3/HP and CNOT4. PEX, hemopexin-like repeats. PHD, PHD-type zinc finger domain. SET, SET domain in Trithorax (TRX). CC, coiled coil domain. Nop25, nucleolar protein 12 (25kDa). P-rich, proline rich domain. CD, chromo domain. CS, chromo-shadow domain. R, Ring type zinc finger E3 ubiquitin ligase domain. RRM, RNA recognition motif. Z, zinc finger domain. F3, $Flag^3$ tag. (B) RT-qPCR analysis of *HSP70B'* mRNA. pFlag3-PEX-myc or the control vector was co-transfected with plasmids overexpressing KMT2E, CBX5, CBX3 or CNOT4 or the control vector into HEK293 cells. Total RNA was prepared at 40 h post-transfection period.

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Figure 6. MMP3-GFP overexpressed with the signal peptide translocated to cellular nuclei. (A) A scheme of MMP3-GFP plasmid construct. P, proline residue. *, nuclear localization signals. (B) Nuclear localization of MMP3-GFP, CBX5/HP1α and histone H3. MMP3-GFP was overexpressed in COS7 cells. Nuclear fraction was separated to soluble, benzonase-treated (Benzo.) and benzonase-resistant (Resist.) fractions, and Western blotting analysis was performed. ctrl, non-transfected control.

Figure 7. A current model of HSP70 gene activation.

In the present study, we showed that MMP3 positively regulate *HSP70* transcription through interaction with HP1/CBX proteins. It has been shown that HSF1 mediates mTOR signal-triggered *HSP70* induction. It has also been shown that HSP70 has a role in protein folding and protein degradation that may lead activation of oncoproteins such as c-Met.

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Figure 1

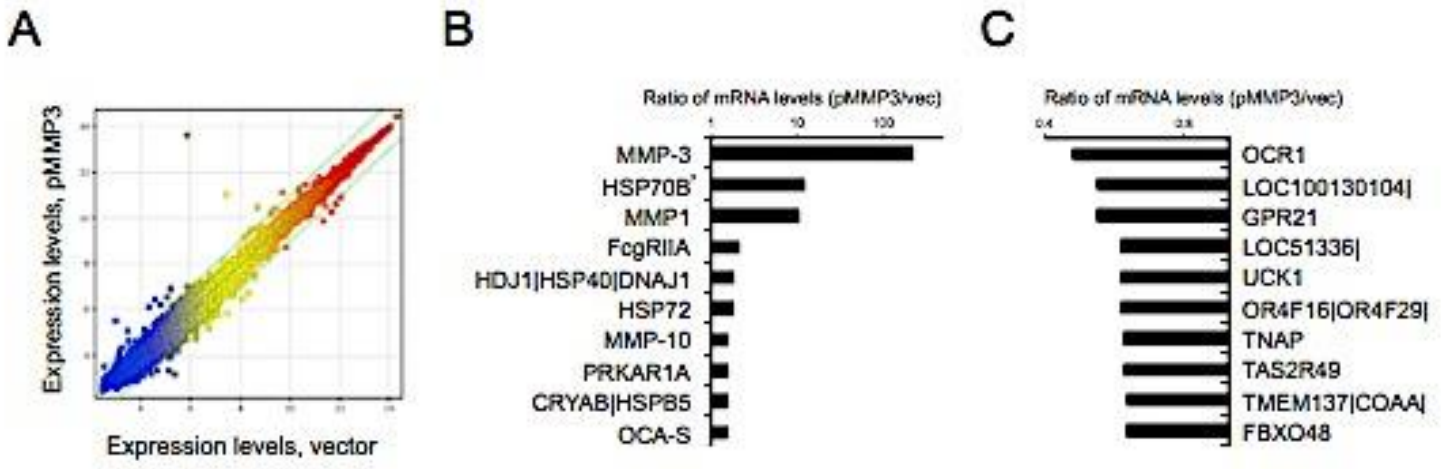
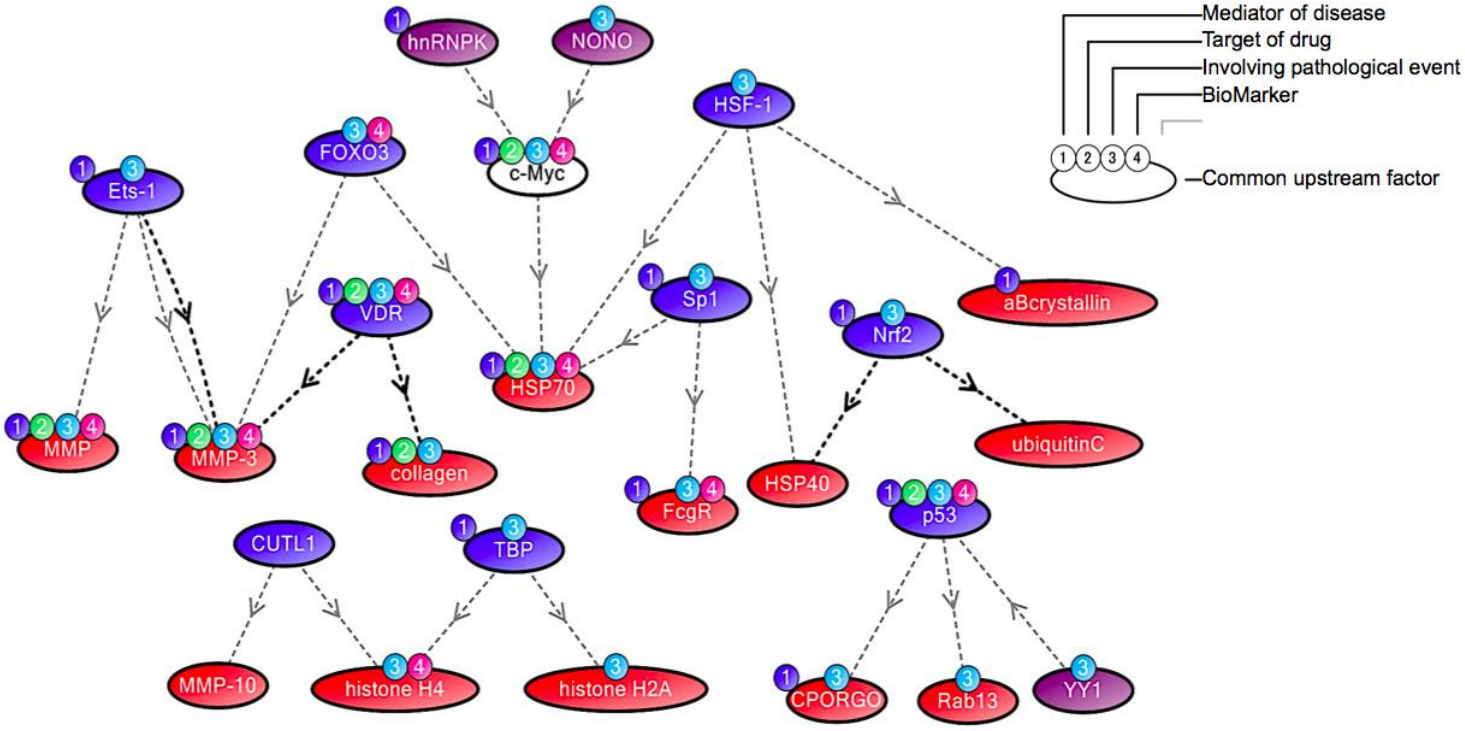


Figure 2



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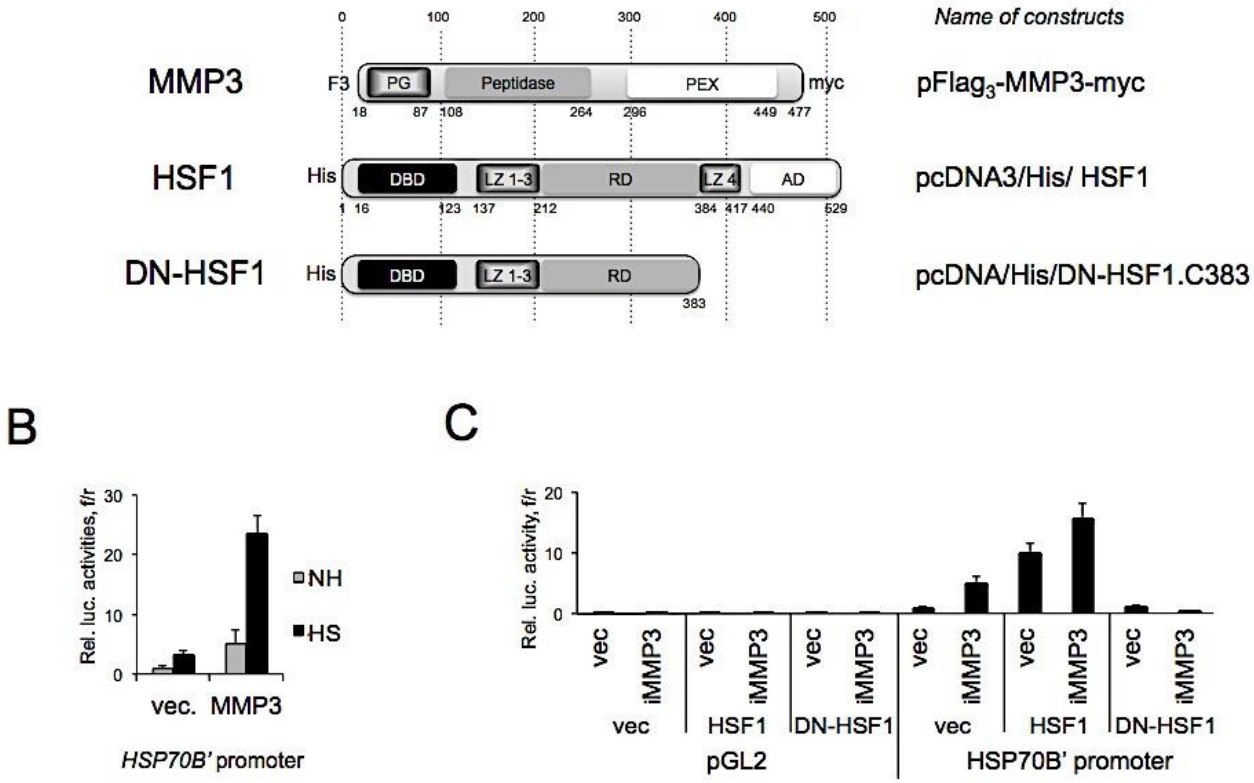
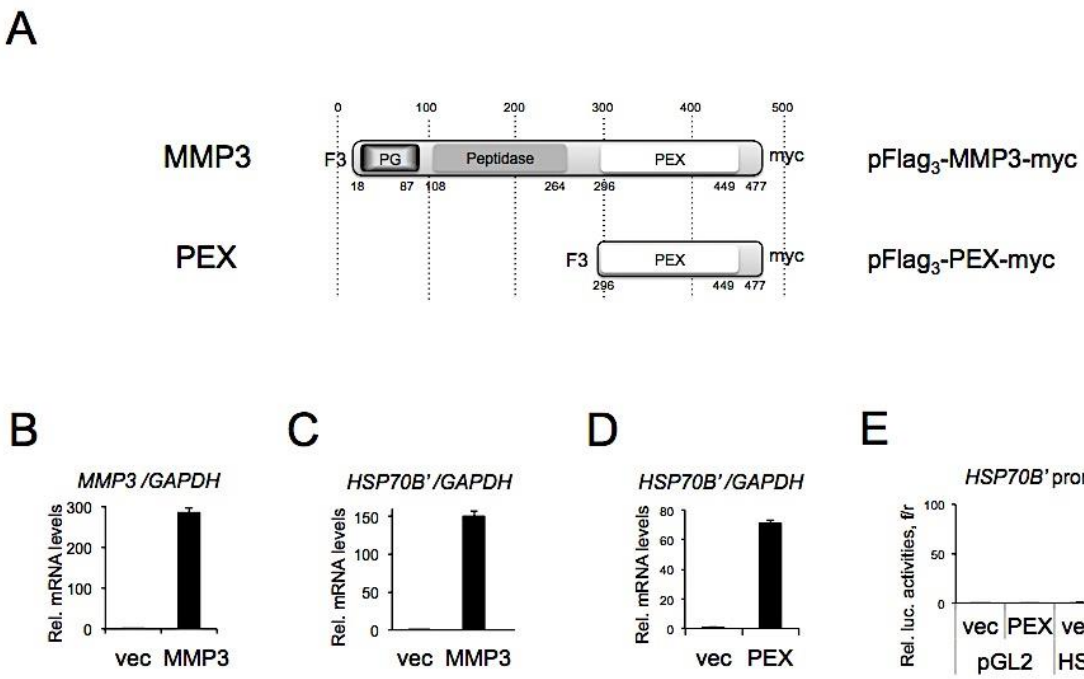


Figure 4



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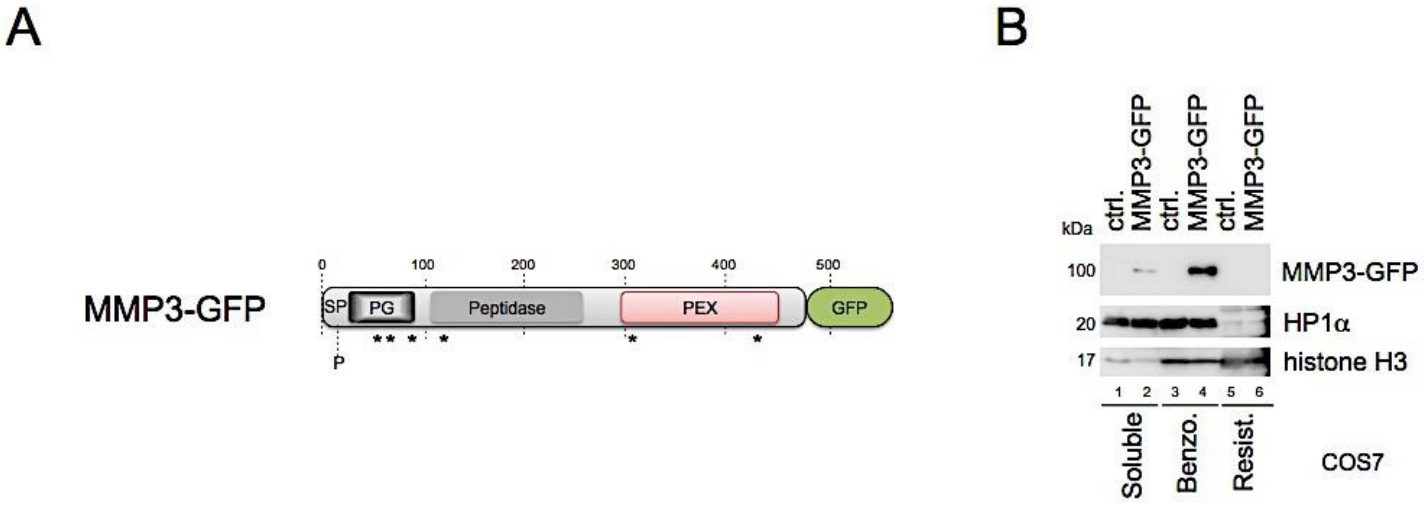

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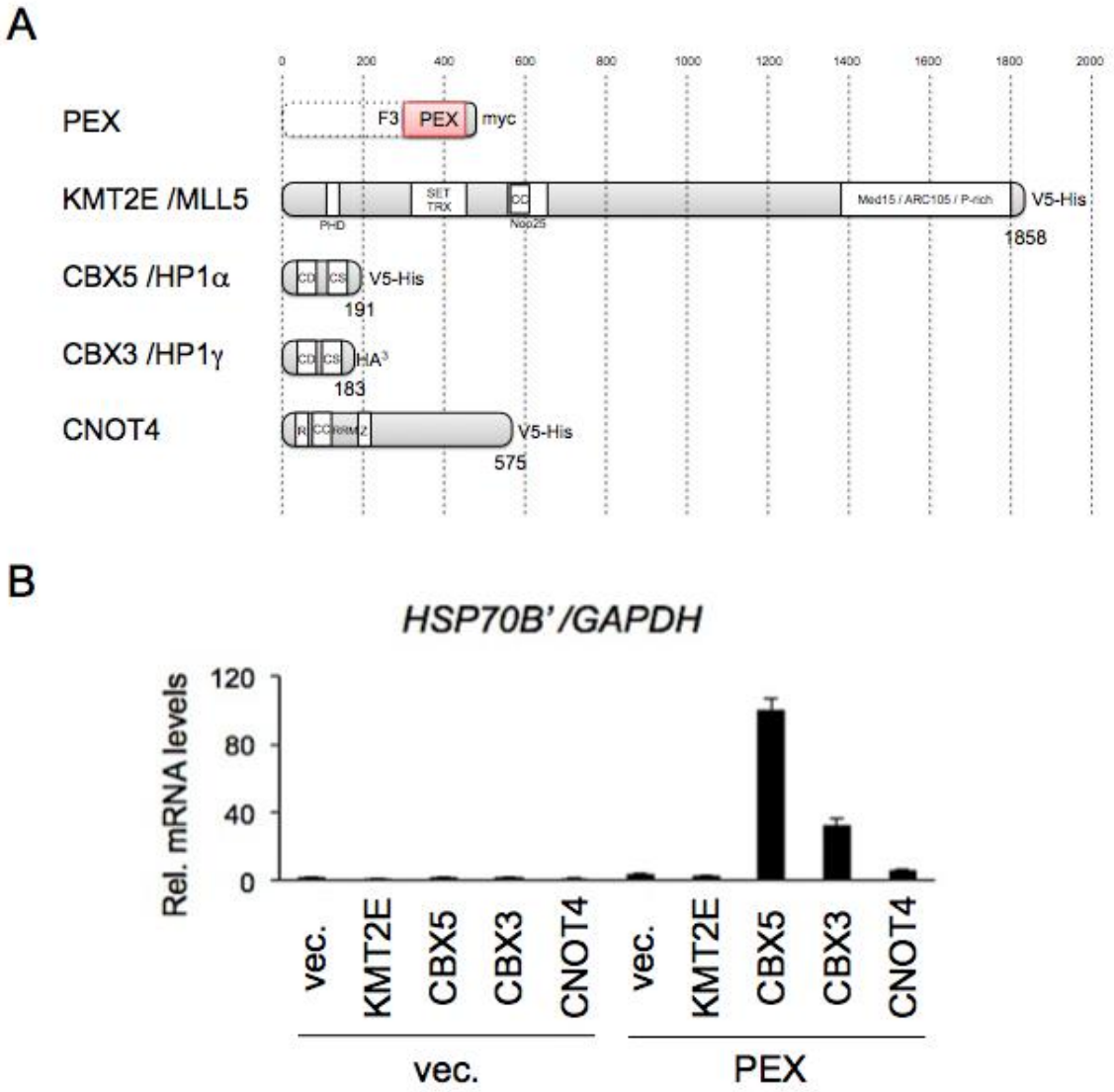
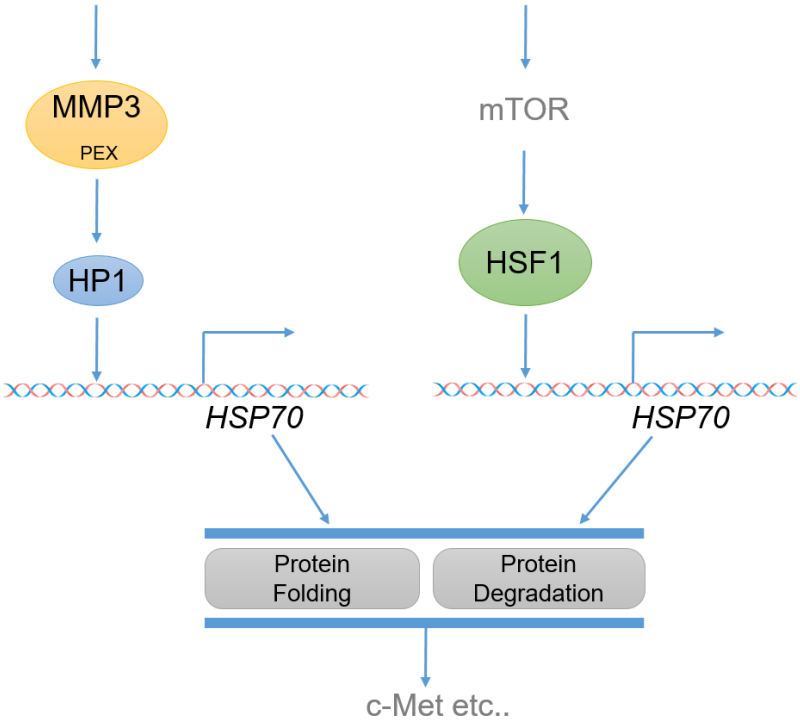


Figure 7



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