



HAL
open science

Signaling to Heme Oxygenase-1 and its Anti-Inflammatory Therapeutic Potential

Ananta Paine, Britta Eiz-Vesper, Rainer Blasczyk, Stephan Immenschuh

► **To cite this version:**

Ananta Paine, Britta Eiz-Vesper, Rainer Blasczyk, Stephan Immenschuh. Signaling to Heme Oxygenase-1 and its Anti-Inflammatory Therapeutic Potential. *Biochemical Pharmacology*, 2010, 80 (12), pp.1895. 10.1016/j.bcp.2010.07.014 . hal-00637151

HAL Id: hal-00637151

<https://hal.science/hal-00637151>

Submitted on 31 Oct 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Signaling to Heme Oxygenase-1 and its
Anti-Inflammatory Therapeutic Potential

Authors: Ananta Paine, Britta Eiz-Vesper, Rainer Blasczyk,
Stephan Immenschuh



PII: S0006-2952(10)00525-3
DOI: doi:10.1016/j.bcp.2010.07.014
Reference: BCP 10647

To appear in: *BCP*

Received date: 5-5-2010
Revised date: 5-7-2010
Accepted date: 8-7-2010

Please cite this article as: Paine A, Eiz-Vesper B, Blasczyk R, Immenschuh S, Signaling to Heme Oxygenase-1 and its Anti-Inflammatory Therapeutic Potential, *Biochemical Pharmacology* (2010), doi:10.1016/j.bcp.2010.07.014

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

REVIEW

1
2
3
4
5 **Signaling to Heme Oxygenase-1 and its Anti-Inflammatory**
6
7
8 **Therapeutic Potential***
9

10
11
12
13 Ananta Paine, Britta Eiz-Vesper, Rainer Blasczyk, Stephan Immenschuh¹
14
15

16
17
18 Institute for Transfusion Medicine, Hannover Medical School, Hannover, Germany
19
20
21
22
23
24

25
26 ¹ To whom correspondence should be addressed:
27

28 Dr. Stephan Immenschuh, MD
29 Institut für Transfusionsmedizin
30 Medizinische Hochschule Hannover
31 Carl-Neuberg-Str. 1
32 30625 Hannover
33 Germany
34 Tel.: 49-511-532-6704/ -9714
35 Fax: 49-511-532-2079
36 e-mail: Immenschuh.Stephan@mh-hannover.de
37
38
39
40
41
42
43
44
45
46
47
48
49

50 * Supported by grant SFB547 A8 from the Deutsche Forschungsgemeinschaft (SI).
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

ABSTRACT

1
2 Heme oxygenase (HO)-1 is the inducible isoform of the first and rate-limiting enzyme of
3
4 heme degradation. Induction of HO-1 protects against the cytotoxicity of oxidative stress and
5
6 apoptotic cell death. More recently, HO-1 has been recognized to have major
7
8 immunomodulatory and anti-inflammatory properties, which have been demonstrated in HO-
9
10 1 knockout mice and a human case of genetic HO-1 deficiency. Beneficial protective effects
11
12 of HO-1 in inflammation are not only mediated via enzymatic degradation of proinflammatory
13
14 free heme, but also via production of the anti-inflammatory compounds bilirubin and carbon
15
16 monoxide. The immunomodulatory role of HO-1 is associated with its cell type-specific
17
18 functions in myeloid cells (including macrophages and monocytes) and in endothelial cells,
19
20 as both cell types are crucially involved in initiating inflammatory responses. This review
21
22 covers the molecular mechanisms and signaling pathways that are involved in HO-1 gene
23
24 expression. In particular, it is discussed how key nuclear factors such as the redox-
25
26 dependent transcriptional activators NF-E2 related factor 2 (Nrf2), NF- κ B and AP-1 along
27
28 with the transcription repressor BTB and CNC homologue 1 (Bach1) mediate inducible HO-1
29
30 gene expression. The role of central pro- and anti-inflammatory cellular signaling cascades
31
32 including p38 MAPK and phosphatidylinositol-3 kinase (PI3K)/Akt in HO-1 regulation is
33
34 highlighted. Finally, we summarize emerging strategies that apply targeted pharmacological
35
36 induction of HO-1 for therapeutic interventions in inflammatory conditions.
37
38
39
40
41
42
43
44
45
46
47
48

KEYWORDS

49 Gene expression, heme oxygenase-1, immunomodulation, inflammation, oxidative stress,
50
51 signaling.
52
53
54
55
56
57
58
59
60
61
62
63
64
65

INTRODUCTION

1
2 Heme oxygenase (HO) catalyzes the first and rate-limiting enzymatic step of heme
3 degradation and produces carbon monoxide (CO), iron and biliverdin [1-3], which is
4 converted into bilirubin (BR) via biliverdin reductase. Two genetically distinct HO isozymes,
5
6 HO-1 and HO-2, are known. HO-2 represents the constitutive non-inducible isoform and is
7
8 primarily expressed in brain and testis [4]. By contrast, the inducible isoform HO-1, which
9
10 exhibits low basal expression levels in most cells and tissues, is highly up-regulated by a
11
12 wide variety of oxidative stress stimuli. Due to its regulatory pattern, induction of HO-1 has
13
14 generally been considered to be an adaptive cellular response against the toxicity of
15
16 oxidative stress [5-10]. More recently, HO-1 has also been recognized to exhibit important
17
18 immunomodulatory and anti-inflammatory functions. A potential link between HO-1 and
19
20 inflammation has initially been shown in an animal model, in which specific up-regulation of
21
22 HO enzyme activity attenuated complement-dependent inflammation [11]. Shortly thereafter,
23
24 it has been demonstrated in a HO-1 knockout mouse model that these animals develop a
25
26 chronic inflammatory disease and are highly vulnerable to an experimental sepsis induced by
27
28 the classical pro-inflammatory mediator endotoxin [12]. Importantly, phenotypical alterations
29
30 in the only known human case of genetic HO-1 deficiency are highly similar to those
31
32 observed in HO-1 knockout mice [13]. By contrast, HO-2 deficient mice have an intact
33
34 immune system, but exhibit major neurological defects [14]. Independently, targeted
35
36 overexpression of HO-1 has been shown to have beneficial effects in various experimental
37
38 animal models of inflammation [2, 15]. Due to the critical role of HO-1 in immunological
39
40 responses [16-18], the mechanisms of its immunomodulatory functions are currently under
41
42 intense investigation.

43
44 In this review, we discuss the current understanding of how HO-1 may mediate its anti-
45
46 inflammatory effects in myeloid and endothelial cells. Moreover, we summarize the
47
48 regulatory role of major transcription factors (TFs) and signaling pathways that govern the
49
50 inducible HO-1 gene expression. Finally, we highlight the therapeutic potential of targeted
51
52 pharmacological induction of HO-1.

1. Cell-specific immunomodulatory functions of HO-1 in myeloid and endothelial cells

1
2 Inflammation is a complex reaction of the immune system in vascularized tissues at sites of
3
4 an infection, toxin exposure or cell injury. Although HO-1 is expressed in all cells and tissues
5
6 the salutary anti-inflammatory effects of HO-1 appear to be critically dependent on its cell
7
8 type-specific functions in myeloid and endothelial cells.
9

1.1. Myeloid cells

10
11
12
13 Myeloid cells comprise monocytes, macrophages and dendritic cells, which play crucial
14
15 regulatory roles in the innate and adaptive immune system [19]. For example, macrophages
16
17 ingest and kill invading microorganisms as a first line of defense and are activated by various
18
19 immunological stimuli such as microbial products and cytokines to initiate inflammatory
20
21 immune responses [20]. In rodent macrophages, HO-1 has been shown to be up-regulated
22
23 by lipopolysaccharide (LPS) [21-23], which then attenuates the expression of various
24
25 proinflammatory genes including cyclooxygenase-2, inducible nitric oxide (NO) synthase
26
27 (iNOS), tumor necrosis factor (TNF)- α or interleukin (IL)-6 [18, 24] (Figure 1). In contrast to
28
29 the LPS-dependent induction of HO-1 in rodent macrophages, gene expression of HO-1 is
30
31 down-regulated by treatment with LPS in human monocytes [25]. More recently, the myeloid
32
33 cell-specific immunomodulatory functions of HO-1 have also been investigated in a
34
35 conditional HO-1 knockout mouse model. Mice with myeloid cell-specific genetic HO-1
36
37 deficiency exhibited a defect of the interferon- β pathway along with pathological immune
38
39 responses in experimentally induced infections and an experimental autoimmune
40
41 encephalomyelitis [26]. Finally, HO-1 is also important for the function of dendritic cells,
42
43 which are the main cell population for antigen-presentation and for initiation of adaptive
44
45 immune responses. Specifically, targeted up-regulation of HO-1 has been shown to modulate
46
47 maturation and cell-specific functions of dendritic cells in human and mouse models [17, 27].
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

1.2. Endothelial cells

1 Endothelial monolayers are intimately linked with inflammation, because they constitute a
2 barrier between the peripheral blood stream and inflamed tissues. The endothelium regulates
3 recruitment and transmigration of immunologically active blood cells such as
4 polymorphonuclear leukocytes and T lymphocytes to the site of an inflammation [28, 29].
5
6 HO-1 has been shown to directly affect the cellular interactions of polymorphonuclear
7 leukocytes with endothelial cells in an *in vivo* rat model, in which increased HO activity down-
8 regulated the adhesion of these cells during experimental oxidative stress conditions [30].
9
10 Independently, major pathological alterations of the endothelium have been observed in HO-
11 1 knockout mice, in which endothelial cells were more susceptible to apoptotic cell death and
12 denudation from the extracellular matrix [31]. Independently, anti-inflammatory endothelial
13 protection via HO-1 has been shown to be mediated via its ability to down-regulate TNF α -
14 induced expression of various adhesion molecules [32, 33]. More recently, it has been
15 reported that HO-1 was involved in the recruitment of endothelial progenitor cells to the site
16 of an experimental vascular injury in various animal models [34].
17
18 Thus, HO-1 counteracts inflammatory reactions via modulation of various endothelial cell
19 functions.
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40

2. Anti-inflammatory functions of HO-1: degradation of proinflammatory free heme and production of the anti-inflammatory compounds BR and CO

41 The mechanisms that mediate the anti-inflammatory effects of HO-1 are not understood in
42 detail. It has been appreciated in recent years, however, that the enzymatic degradation of
43 proinflammatory free heme and the production of the anti-inflammatory compounds
44 biliverdin/BR and CO may play major roles to counteract inflammatory reactions.
45
46
47
48
49
50
51
52

53 Heme consists of a tetrapyrrole ring with a central iron ion and is an abundant compound in
54 mammals with contradictory biological functions. On the one hand, heme plays a
55 physiological role for oxygen and mitochondrial electron transport as an essential prosthetic
56 group of hemoglobin, myoglobin and cytochromes [35, 36]. On the other hand, non-protein
57
58
59
60
61
62
63
64
65

1 bound free heme is highly toxic as it may cause oxidative stress. Due to the prooxidant
2 properties of free heme, which have been shown in various animal and cell culture models,
3 the enzymatic synthesis and degradation of this molecule is tightly controlled [35, 37, 38].
4 More recently, free heme has also been shown to have proinflammatory properties [10, 15,
5 39]. For example, heme has been demonstrated to be responsible for the increased influx of
6 leukocytes into organs during an experimental inflammation *in vivo* [40]. Detrimental
7 proinflammatory effects of free heme have also been shown in an animal model of
8 experimental cerebral malaria, in which the heme-dependent detrimental effects were more
9 pronounced in HO-1 deficient mice [41]. Thus, it is conceivable that the enzymatic
10 degradation of proinflammatory free heme via HO-1 plays a critical role for the anti-
11 inflammatory functions of HO-1 [10, 15, 37-39].

12 The role of BR as a beneficial compound with potent antioxidant and anti-inflammatory
13 effects has only been appreciated in recent years [42, 43]. Protection against experimental
14 inflammation via HO-1-derived biliverdin has been shown in animal models of
15 proinflammatory cardiovascular [30] and gastrointestinal disorders [44]. Independently,
16 beneficial effects of BR have directly been implicated in the protection against endothelial
17 activation and dysfunction in human aortic endothelial cells [45]. Interestingly, BR has also
18 been suggested to specifically reduce leukocyte transmigration to the site of an experimental
19 inflammation via interaction with adhesion molecules [46].

20 Although CO is generally considered a toxic gas, it has been recognized to have major
21 physiological functions as a signaling molecule [3, 47, 48]. Specifically, HO-1-derived CO has
22 been shown to be involved in the regulation of apoptosis, vasodilation and inflammation. In
23 an early report on the potential protective effects of this gas, administration of exogenous CO
24 blocked the LPS-induced production of proinflammatory cytokines via modulation of p38
25 MAP kinase [24]. Similar to the signaling gas NO, CO up-regulates the production of cGMP
26 and this mechanism has been implicated in other functions of CO such as vasodilation and
27 blockage of smooth muscle cell proliferation. Major potential for future therapeutic

1 applications may have CO-releasing molecules (CORMs), which are compounds that deliver
2 CO to its target sites without the toxicity of gaseous CO [49, 50].
3

4 In summary, HO-1 counteracts inflammatory responses via metabolic conversion of
5 proinflammatory free heme and production of the anti-inflammatory compounds BR and CO.
6
7
8
9

10 **3. Redox-dependent TFs mediate the inducible HO-1 gene expression**

11 Targeted modulation of HO-1 for potential anti-inflammatory therapeutic interventions not
12 only requires detailed knowledge of the immunomodulatory effects of HO-1. To achieve this
13 goal, it is also necessary to precisely understand the mechanisms that regulate HO-1 gene
14 expression.
15
16
17
18
19
20
21
22
23

24 HO-1 is induced by a plethora of physiological and pathological stimuli including oxidative
25 stress signals, cytokines, bacterial compounds and growth factors. HO-1 expression is
26 primarily regulated on the transcriptional level and multiple *cis*-acting regulatory elements
27 (REs) of the HO-1 promoter have been shown to mediate the basal and inducible HO-1 gene
28 expression in different species (reviewed in [2, 8, 9, 51]). Two upstream enhancer regions,
29 which are termed E1 and E2, play major functional roles for redox-dependent induction of
30 HO-1 [52, 53]. Both E1 and E2 enhancer regions contain several antioxidant response
31 elements (AREs), which have also been identified in the promoters of other stress-inducible
32 antioxidant and phase 2 detoxifying genes [54, 55]. An important difference between the
33 rodent and human HO-1 genes with major biological relevance is a GT-microsatellite
34 polymorphism, which is localized in the proximal human HO-1 gene promoter region. Lower
35 numbers of GT repeats within this polymorphic sequence have been associated with higher
36 inducibility of HO-1 gene expression in response to stress stimuli [56] and individuals with
37 this allele seem to be protected against cardiovascular disorders (reviewed in [57]).
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54

55 In the following, we highlight the critical role of the major redox-dependent TFs NF-E2-related
56 factor 2 (Nrf2), BTB and CNC homologue 1 (Bach1), NF- κ B and AP-1 in regulating the
57 inducible HO-1 gene expression.
58
59
60
61
62
63
64
65

3.1. The Keap1/Nrf2 system

Redox-dependent transcriptional induction of HO-1 is primarily mediated through the cap'n'collar (CNC) TF Nrf2 [58], which was initially identified while screening for proteins that interact with the NF-E2 binding motif [59]. Nrf2 forms heterodimers with small Maf proteins and up-regulates a program of inducible protective genes via interaction with AREs [55, 60]. Activation of Nrf2 by oxidative stress is mainly controlled by the cytosolic inhibitor Kelch-like ECH-associated protein 1 (Keap1)[61, 62], also termed inhibitor of Nrf2 [63](Figure 2). Numerous prooxidant stimuli cause dissociation of Nrf2 from Keap1, which then permits subsequent nuclear translocation of Nrf2 [63, 64]. In a zebrafish model it has been demonstrated that various chemicals activate Nrf2 in a compound-specific manner via the modulation of various regulatory sites of Keap1 [65]. More recently, the Keap1/Nrf2 pathway has also been shown to be activated by the regulator protein p62 in experimental conditions that cause autophagy [66]. Thus, the Keap1/Nrf2 system appears to be a central sensor for a broad spectrum of unfavourable cellular conditions.

It is important to point out, that the Keap1/Nrf2 module may not only be regulated by prooxidant stimuli, but also by stress-independent signals such as glycogen synthase kinase (GSK)-3 β -dependent phosphorylation (Figure 3). Finally, it is not clear whether Nrf2-dependent induction of HO-1 is part of a general Nrf2-regulated antioxidant response that includes other Keap1/Nrf2-regulated genes such as NAD(P)H:oxidoreductase or thioredoxin reductase-1. A potential mechanism that could mediate Nrf2-specific induction of HO-1 involves the *brahma-related gene 1* (BRG1). BRG1 has been shown to be necessary for the specific recruitment of Nrf2 to the promoter of the HO-1 gene, but not to that of other phase 2 detoxifying genes [67].

In conclusion, the redox-dependent Keap1/Nrf2 system plays a central role for HO-1 induction in response to oxidativ stress.

3.3. The transcription repressor Bach1

1
2 The transcription repressor Bach1 has been recognized to be a key regulator of the inducible
3
4 HO-1 gene expression (Figure 2). Bach1 was initially identified as a regulator of globin gene
5
6 expression in erythroid cells [68]. Similar to Nrf2, Bach1 belongs to the CNC family of TFs
7
8 and forms heterodimers with small Maf proteins that bind to Maf recognition elements [60,
9
10 69] such as AREs of the HO-1 promoter. In contrast to Nrf2, Bach1 has six heme regulatory
11
12 motifs, which are crucial for its regulatory functions. Bach1 has initially been shown to
13
14 repress HO-1 gene expression in the presence of low levels of intracellular heme. When
15
16 intracellular heme levels are elevated, Bach1 changes its conformation and dissociates from
17
18 the HO-1 promoter, allowing Nrf2 to bind to the ARE and to activate HO-1 gene expression
19
20 [70] (Figure 2). More recently, heme has also been shown to control cellular Bach1 protein
21
22 levels via a mechanism that involves proteasomal degradation of this protein [71]. As free
23
24 heme exhibits major proinflammatory effects, it is conceivable that Bach1 might play a
25
26 regulatory role in inflammation. In line with this notion, a regulatory link between Bach1 and
27
28 the proinflammatory cytokine IL-6 has recently been elucidated in Bach1 knockout mice in
29
30 the context of hyperoxic lung injury [72].

31
32 Bach1 does not only regulate HO-1 gene expression by heme, but also by other prooxidant
33
34 compounds such as cadmium [73], diamide [74] and sodium arsenite [75]. More recently, the
35
36 regulatory role of Bach1 in stress-dependent HO-1 induction has also been shown to be
37
38 dependent on the differentiation status of keratinocytes in cell culture [76].

39
40 The interplay between Bach1 and Nrf2 to regulate HO-1 gene expression is discussed
41
42 controversially. As an example, sodium arsenite has been shown to cause Bach1-specific
43
44 HO-1 induction independent from Nrf2, whereas sodium arsenite-dependent regulation via
45
46 this mechanism was not observed for other ARE-regulated genes such as thioredoxin
47
48 reductase-1 [75]. Similarly, it has recently been demonstrated that knockdown of Bach1 in
49
50 human keratinocytes specifically up-regulated gene expression of HO-1, but not that of other
51
52 Nrf2-regulated genes [77]. In contrast, other investigators have reported that Bach1 induced
53
54 HO-1 gene expression in coordination with a subset of other Nrf2-regulated genes [78, 79].
55
56
57
58
59
60
61
62
63
64
65

1
2 In conclusion, the interplay of Bach1 and Nrf2 is crucial for the redox-dependent HO-1
3 induction and might give this system a high range of plasticity to adapt to adverse cellular
4 conditions.
5

6 7 8 **3.4. NF- κ B** 9

10 The NF- κ B/Rel family of proteins comprises several TFs that regulate the inducible gene
11 expression of various immunological and antioxidant protective responses including the up-
12 regulation of major proinflammatory cytokines, adhesion molecules and antioxidant stress
13 proteins [80, 81]. Under basal conditions NF- κ B is contained in the cytoplasm by inhibitor of
14 NF- κ B (I- κ B). In response to multiple signals the regulatory NF- κ B subunits p50 and p65
15 dissociate from I- κ B and subsequently translocate to the nucleus [82, 83]. Although NF- κ B
16 has been shown to be induced by stimuli that are also known to up-regulate HO-1 gene
17 expression, the role of this TF in HO-1 gene regulation has been discussed controversially.
18 This might be explained by the fact that functional κ B-elements have remained elusive for a
19 long while, because indirect approaches including computer-based sequence predictions,
20 treatment with pharmacological NF- κ B inhibitors and dominant negative mutants of I- κ B have
21 been applied in most studies.
22

23 In two recent reports functional binding sites for NF- κ B of the promoters of the rat and mouse
24 HO-1 genes have been identified. A κ B element of the proximal rat HO-1 gene promoter
25 region has been shown to control HO-1 up-regulation by the phorbol ester phorbol myristate
26 acetate (PMA), which is an activator of macrophages. PMA-dependent up-regulation of HO-1
27 gene expression was not observed in cells from mice, which were deficient for the NF- κ B
28 subunit p65 and was mediated via an I- κ B-kinase-independent pathway [84]. Moreover, Li
29 and colleagues have described a functional κ B element in the mouse HO-1 promoter. These
30 authors have shown that a mechanism involving the NF- κ B subunits p50 and p65 as well as
31 the inducible NO-synthase mediated HO-1 up-regulation *in vivo* [85].
32

33 In summary, NF- κ B appears to be directly involved in the induction of HO-1 gene expression.
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

3.5. Activating protein-1 (AP-1)

1
2 The TF AP-1 is composed of structurally and functionally related members of the Jun (c-Jun,
3 JunB and JunD), Fos (c-Fos, FosB, Fra1 and Fra2) and activating TF (ATF) protein families.
4
5 Dimers of these proteins regulate gene expression via interaction with AP-1 sites, which are
6
7 also known as TPA-responsive elements (TREs). Inducible gene expression via AP-1 has
8
9 been shown to be involved in a diverse range of cellular responses including immunological
10
11 and antioxidant stress responses [86, 87]. Similar to NF- κ B, AP-1 is up-regulated by a wide
12
13 variety of prooxidant and pro-inflammatory stimuli. Alam and colleagues have initially
14
15 reported that AP-1 played a crucial role for the induction of the mouse HO-1 gene [53, 88].
16
17 Subsequently, various functional AP-1 sites, which mediated inducer-dependent gene
18
19 expression of HO-1, have been identified in the promoter regions of the rat and human HO-1
20
21 genes [51, 88-91]. Elucidation of the molecular mechanisms that are involved in AP-1-
22
23 dependent HO-1 gene regulation has turned out to be challenging for two major reasons.
24
25 First, the classical AP-1 (TGATGCA) site is contained in the consensus sequence of AREs
26
27 (TGCTGAGTCA), which are localized in the E1 and E2 regions of the HO-1 gene promoter
28
29 and serve as major target sites for Nrf2 [58]. Cross-talk of Nrf2 with members of the AP-1
30
31 family, however, appears to be highly complex. As an example, c-Jun has recently been
32
33 shown to directly interact with Nrf2 to activate the expression of the ARE-regulated genes
34
35 NAD(P)H:quinone reductase and glutamate-cysteine ligase catalytic subunit by the chemical
36
37 compound 4-hydroxy-2-nonenal [92]. Moreover, others have shown that Nrf2 might indirectly
38
39 regulate the inducible expression of the glutamate-cysteine ligase catalytic subunit gene via
40
41 activation of AP-1 [93]. Second, AP-1 sites in the HO-1 promoter mediate inducer-dependent
42
43 HO-1 gene expression via mechanisms that involve synergistic cooperation of AP-1 with
44
45 other TFs such as USF2 or SP-1. This has been shown for regulation of the rodent and
46
47 human HO-1 genes in various cell culture models [94-97].
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

In summary, we have highlighted the role of various redox-dependent TFs that mediate the complex regulation of inducible HO-1 gene expression. Detailed overviews on this issue have also been given by others [2, 8, 51].

4. Signaling cascades that mediate HO-1 gene regulation

1
2 In general, activation of TFs is regulated by intracellular signaling cascades, which are
3 controlled by modules of kinases/phosphatases and redox reactions. In the following, we
4 highlight major signalling cascades that mediate HO-1 induction and are involved in the
5 regulation of inflammatory immune responses.
6
7
8
9

4.1. p38 MAPK

10
11
12
13 It has been known for many years that activation of MAPKs plays a central role for the
14 induction of HO-1 gene expression [51] (Figure 3). Three major subfamilies of MAPK are
15 known: extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38
16 MAPK [98]. While ERK is primarily considered to be activated in response to hormones and
17 growth factors, JNK and p38 are primarily induced by stress-related stimuli [98, 99]. Due to
18 space limitations, it is not intended to give a comprehensive overview on how various MAPKs
19 might mediate HO-1 gene regulation, but rather discuss the specific role of p38 MAPK for
20 HO-1 regulation.
21
22
23
24
25
26
27
28
29
30
31

32
33 Similar to HO-1, p38 MAPK has been shown to be involved in antioxidant and anti-
34 inflammatory responses. Not surprisingly, gene expression of HO-1 and p38 MAPK are
35 activated by the same or similar stimuli. Numerous reports have demonstrated that inhibition
36 of p38 activity by specific small molecule inhibitors, dominant negative mutants or gene
37 silencing strategies blocked HO-1 induction in response to multiple stimuli [2, 9, 51] (Figure
38 3). Of note, the α and β isoforms of p38 play counter-regulatory roles to the p38 γ and δ
39 isoforms, which has been demonstrated for sodium arsenite- and LPS-dependent induction
40 of HO-1 [100, 101]. Unexpectedly and contradictory to what is generally thought on the
41 activating role of p38 MAPK on HO-1 gene expression, inhibition of p38 α has been
42 demonstrated to up-regulate HO-1 gene expression in human hepatoma cells via interaction
43 with the TF Nrf2 [102, 103]. These findings have essentially been confirmed in an
44 independent report, in which pharmacological inhibition and genetic deficiency of p38 α also
45 up-regulated HO-1 gene expression [104]. Increased levels of HO-1 gene expression in
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 p38 α -deficient cells might be explained by the fact that these cells contain increased levels of
2 intracellular ROS as compared to wild type cells [105]. Because p38 α is a sensor of ROS,
3
4 cross-talk between HO-1 and p38 α may have additional yet unknown functions in the context
5
6 of oxidative stress and inflammation. It is also important to point out that p38 α is not only an
7
8 upstream regulator of HO-1, but that p38 α is also a downstream target of HO-1. Silva and
9
10 colleagues have demonstrated that increased HO-1 activity degraded p38 α in endothelial
11
12 cells suggesting that these two molecules might form a physiological relevant feedback loop
13
14 to regulate apoptosis [106].
15
16

17
18 In summary, p38 and HO-1 make up a closely linked signaling module with major regulatory
19
20 functions in antioxidant and anti-inflammatory cellular responses.
21
22

23 24 25 **4.2. The phosphatidylinositol-3 kinase (PI3K)/Akt pathway**

26
27 PI3K/Akt is an anti-apoptotic survival pathway and is regulated by a number of receptor-
28
29 dependent mechanisms that are activated by growth factors and cytokines [107]. Moreover,
30
31 in models of PI3K genetic deficiency an essential role of this kinase has been implicated in
32
33 the regulation of inflammatory reactions [108]. Accumulating experimental evidence has
34
35 indicated that activation of PI3K/Akt not only up-regulates HO-1 gene expression, but that the
36
37 protective effects of this signaling cascade might be intimately linked with the salutary effects
38
39 of HO-1 [109]. HO-1 gene expression has been shown to be up-regulated via PI3K/Akt in
40
41 immunological cells in response to various signals such as prostaglandins or the
42
43 pharmacological compounds [2, 94, 109, 110]. A more recent report has shown that HO-1
44
45 induction was mediated via activation of this signaling cascade by a mitochondrial redox-
46
47 dependent pathway in vascular endothelial cells [111]. Finally, it has been suggested that
48
49 PI3K/Akt and GSK3 β may have counter-regulatory functions in HO-1 gene regulation. The
50
51 complex interplay between these two kinases appears to involve mechanisms that control
52
53 the nuclear localization of Nrf2 and Bach1 [112, 113] (Figure 3). Details on the underlying
54
55 regulatory mechanisms, however, are largely unknown.
56
57
58
59
60
61
62
63
64
65

1
2 In conclusion, the PI3K/Akt cascade is a key mediator of HO-1 induction in response to
3 cytokines and growth factors.
4
5

6 **4.3. IL-10 and the Jak-STAT pathway**

7
8 The anti-inflammatory cytokine IL-10, which is a major inhibitor of activated macrophages
9 and dendritic cells, has consistently been shown to induce HO-1 gene expression as
10 reported by Lee and Chau [114] as well as by others [115-117]. Although the signaling
11 cascades that couple HO-1 gene expression to the IL-10 receptor are not clear, activation of
12 STAT3 has repeatedly been suggested to be involved in this regulatory pathway [116]. Jak-
13 STAT signaling plays a key role in the immune system and mediates major cytokine-
14 activated pathways. In endothelial cells STAT3 has been shown to be necessary to mediate
15 the HO-1 dependent protection against hyperoxic lung injury [118]. Functional STAT3
16 elements have recently been identified in the promoter regions of the rat [119] and human
17 [120] HO-1 genes. Interestingly, a positive feedback circuit between IL-10 and HO-1 has
18 been shown to be functional, which might amplify the anti-inflammatory effects of IL-10 in
19 LPS-stimulated macrophages [114].
20
21

22 Taken together, the IL-10 pathway is an important regulator of HO-1 gene expression in
23 mononuclear cells.
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41

42 **4.4. The toll-like receptor (TLR)-4 pathway**

43
44 HO-1 induction by LPS is mediated via (a) TLR-4 coupled pathway(s) and has been
45 extensively studied in macrophages [21, 23]. Interestingly, Figueiredo and colleagues have
46 recently demonstrated that heme led to HO-1 gene expression via direct interaction with
47 TLR-4 and suggested that this interaction could at least partially explain the proinflammatory
48 LPS-like effects of heme [121]. HO-1 gene expression by LPS in macrophages is not only
49 induced via a TLR-4-dependent mechanism, but increased HO-1 activity has also been
50 shown to have inhibitory effects on intracellular signaling, that is initiated by TLR-4-activation.
51 The inhibitory effect of HO-1 on TLR-4 signaling could be regulated via HO-1-derived CO,
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

which blocked translocation of TLR-4 into lipid rafts [122] and/or the interaction of TLR-4 with the structural membrane protein caveolin-1 [123]. This regulatory interplay between TLR-4 and HO-1 appears to form a negative feedback loop, which might inhibit excessive activation of macrophages by LPS.

Taken together, activation of the central proinflammatory TLR-4 cascade leads to HO-1 gene activation and a negative feedback circuit in macrophages might be of major significance for the regulation of inflammatory responses.

5. Pharmacological induction of HO-1 as an anti-inflammatory therapeutic target

A promising near term approach to apply HO-1 for therapeutic interventions is the targeted induction of this inducible enzyme via pharmacological compounds. In the following, we discuss how pharmacological induction of HO-1 might be applicable for specific anti-inflammatory therapeutic interventions.

Metalloporphyrins such as cobalt protoporphyrin IX, which are prototypical inducers of HO-1 and are commonly used in experimental cell culture and animal models, do not seem to be applicable for clinical interventions, because they lack cell-specificity and are severely toxic. Similarly, the HO substrate heme, which is one of the most potent inducers of HO-1 and has been approved for the treatment of acute intermittent porphyria (heme arginate), only has limited potential for the treatment of inflammatory disorders. By contrast, a growing number of currently available pharmacologic compounds, which induce HO-1 and are applied in standard therapies, might be useful for clinical interventions in inflammatory disorders. As an example, statins, which have initially been introduced to prevent atherosclerosis via their cholesterol-lowering effects, have recently also been recognized to exhibit anti-inflammatory effects via HO-1 induction [124, 125]. Moreover, treatment with 5-aminosalicylic acid (5-ASA), which is one of the pharmacologic standard therapies of inflammatory bowel disease, has been shown to mediate its protective anti-inflammatory effects at least in part through up-regulation of HO-1 in an animal model of colitis [126]. Finally, various polyphenols, which are a group of antioxidant compounds and are currently investigated for their anti-

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

inflammatory and anticancer activities, have been shown to provide anti-inflammatory protection via the induction of HO-1 [127].

Due to the cell-type specific immunomodulatory effects of HO-1, targeted up-regulation of HO-1 in myeloid and endothelial cells seems to be a straightforward anti-inflammatory therapeutic option. In fact, accumulating evidence has demonstrated that specific HO-1 induction in these cells protects against inflammatory reactions. As an example, it has recently been demonstrated in an *in vivo* rat model that cell-specific up-regulation of HO-1 in liver tissue macrophages via the cytokine adiponectin protects against experimental ethanol-dependent inflammation [117]. Other compounds, which have been shown to mediate myeloid cell-specific induction of HO-1, are the cardiovascular hormone atrial natriuretic peptide [128] and the clinically applied antiprotease compound 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF) [94]. Clearly, these compounds might be applicable to specifically induce HO-1 in myeloid cells for therapeutic interventions. In endothelial cells, quercetin and theaflavin have been shown to provide specific HO-1-dependent anti-inflammatory protection in an ApoE knockout mouse model of atherosclerosis [129]. Moreover, it has recently been demonstrated that the nonsteroidal anti-inflammatory drug celecoxib provided specific anti-inflammatory effects in endothelial cells via induction of HO-1 [111].

Finally, it is important to point out that targeted up-regulation of HO-1 has failed to provide anti-inflammatory protection, when induced after the onset of inflammation. This has been shown in animal models of inflammatory bowel disease and pancreatitis, respectively. In either case HO-1 was only protective when induced before the onset of experimental inflammation [130, 131]. These findings indicate that anti-inflammatory protection via HO-1 induction is questionable in established inflammation, but might be useful as a preventive measure.

In summary, targeted HO-1 induction in myeloid and endothelial cells has major anti-inflammatory therapeutic potential. Therefore, identification and characterization of

1 pharmacological compounds that induce HO-1 in a cell-specific and cell context-specific
2 manner deserve further attention.
3
4
5

6 **6. Conclusions**

- 7
8
9
10
11 1. HO-1 plays key immunomodulatory and anti-inflammatory roles via its cell type-
12 specific effects in myeloid and endothelial cells.
13
14
15
16
17
- 18 2. Critical functions of HO-1 are degradation of proinflammatory free heme and
19 enzymatic production of the anti-inflammatory compounds CO and BR.
20
21
22
23
- 24 3. The inducible HO-1 gene regulation is mediated via an interplay of redox-
25 dependent activating TFs and the transcription repressor Bach1, which are under
26 the control of a complex network of signaling cascades.
27
28
29
30
- 31 4. Targeted induction of HO-1 in myeloid and endothelial cells has major therapeutic
32 potential for the treatment of inflammatory disorders.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

REFERENCES

- [1] Tenhunen R, Marver HS, Schmid R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. *Proc Natl Acad Sci USA* 1968;61:748-55.
- [2] Ryter SW, Alam J, Choi AM. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. *Physiol Rev* 2006;86:583-650.
- [3] Maines MD. The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 1997;37:517-54.
- [4] Trakshel GM, Kutty RK, Maines MD. Purification and characterization of the major constitutive form of testicular heme oxygenase. The noninducible isoform. *J Biol Chem* 1986;261:11131-7.
- [5] Keyse SM, Applegate LA, Tromvoukis Y, Tyrrell RM. Oxidant stress leads to transcriptional activation of the human heme oxygenase gene in cultured skin fibroblasts. *Mol Cell Biol* 1990;10:4967-9.
- [6] Vile GF, Basu-Modak S, Waltner C, Tyrrell RM. Heme oxygenase 1 mediates an adaptive response to oxidative stress in human skin fibroblasts. *Proc Natl Acad Sci U S A* 1994;91:2607-10.
- [7] Otterbein LE, Choi AM. Heme oxygenase: colors of defense against cellular stress. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L1029-37.
- [8] Choi AM, Alam J. Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am J Respir Cell Mol Biol* 1996;15:9-19.
- [9] Immenschuh S, Ramadori G. Gene regulation of heme oxygenase-1 as a therapeutic target. *Biochem Pharmacol* 2000;60:1121-8.
- [10] Gozzelino R, Jeney V, Soares MP. Mechanisms of cell protection by heme oxygenase-1. *Annu Rev Pharmacol Toxicol* 2010;50:323-54.
- [11] Willis D, Moore AR, Frederick R, Willoughby DA. Heme oxygenase: a novel target for the modulation of the inflammatory response. *Nat Med* 1996;2:87-90.
- [12] Poss KD, Tonegawa S. Reduced stress defense in heme oxygenase 1-deficient cells. *Proc Natl Acad Sci U S A* 1997;94:10925-30.
- [13] Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma T, et al. Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. *J Clin Invest* 1999;103:129-35.
- [14] Burnett AL, Johns DG, Kriegsfeld LJ, Klein SL, Calvin DC, Demas GE, et al. Ejaculatory abnormalities in mice with targeted disruption of the gene for heme oxygenase-2. *Nat Med* 1998;4:84-7.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [15] Soares MP, Marguti I, Cunha A, Larsen R. Immunoregulatory effects of HO-1: how does it work? *Curr Opin Pharmacol* 2009;9:482-9.
- [16] George JF, Braun A, Brusko TM, Joseph R, Bolisetty S, Wasserfall CH, et al. Suppression by CD4+CD25+ regulatory T cells is dependent on expression of heme oxygenase-1 in antigen-presenting cells. *Am J Pathol* 2008;173:154-60.
- [17] Remy S, Blancou P, Tesson L, Tardif V, Brion R, Royer PJ, et al. Carbon monoxide inhibits TLR-induced dendritic cell immunogenicity. *J Immunol* 2009;182:1877-84.
- [18] Kapturczak MH, Wasserfall C, Brusko T, Campbell-Thompson M, Ellis TM, Atkinson MA, et al. Heme oxygenase-1 modulates early inflammatory responses: evidence from the heme oxygenase-1-deficient mouse. *Am J Pathol* 2004;165:1045-53.
- [19] Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. *Science* 2010;327:656-61.
- [20] Zhang X, Mosser DM. Macrophage activation by endogenous danger signals. *J Pathol* 2008;214:161-78.
- [21] Otterbein L, Chin YB, Otterbein SL, Lowe VC, Fessler HE, Choi AMK. Mechanism of hemoglobin-induced protection against endotoxemia in rats: a ferritin-independent pathway. *Am J Physiol* 1997;272:L268-L75.
- [22] Bauer I, Wanner GA, Rensing H, Alte C, Miescher EA, Wolf B, et al. Expression pattern of heme oxygenase isoenzymes 1 and 2 in normal and stress-exposed rat liver. *Hepatology* 1998;27:829-38.
- [23] Immenschuh S, Tan M, Ramadori G. Nitric oxide mediates the lipopolysaccharide dependent upregulation of the heme oxygenase-1 gene expression in cultured rat Kupffer cells. *J Hepatol* 1999;30:61-9.
- [24] Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, et al. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat Med* 2000;6:422-8.
- [25] Miyazaki T, Kirino Y, Takeno M, Samukawa S, Hama M, Tanaka M, et al. Expression of heme oxygenase-1 in human leukemic cells and its regulation by transcriptional repressor Bach1. *Cancer Sci* 2010.
- [26] Tzima S, Victoratos P, Kranidioti K, Alexiou M, Kollias G. Myeloid heme oxygenase-1 regulates innate immunity and autoimmunity by modulating IFN-beta production. *J Exp Med* 2009;206:1167-79.
- [27] Park DJ, Agarwal A, George JF. Heme Oxygenase-1 Expression in Murine Dendritic Cell Subpopulations. Effect on CD8+ Dendritic Cell Differentiation in Vivo. *Am J Pathol* 2010.
- [28] Muller WA. Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response. *Trends Immunol* 2003;24:327-34.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [29] Cook-Mills JM, Deem TL. Active participation of endothelial cells in inflammation. *J Leukoc Biol* 2005;77:487-95.
- [30] Hayashi S, Takamiya R, Yamaguchi T, Matsumoto K, Tojo SJ, Tamatani T, et al. Induction of heme oxygenase-1 suppresses venular leukocyte adhesion elicited by oxidative stress: role of bilirubin generated by the enzyme. *Circ Res* 1999;85:663-71.
- [31] True AL, Olive M, Boehm M, San H, Westrick RJ, Raghavachari N, et al. Heme oxygenase-1 deficiency accelerates formation of arterial thrombosis through oxidative damage to the endothelium, which is rescued by inhaled carbon monoxide. *Circ Res* 2007;101:893-901.
- [32] Liu X, Spolarics Z. Methemoglobin is a potent activator of endothelial cells by stimulating IL-6 and IL-8 production and E-selectin membrane expression. *Am J Physiol Cell Physiol* 2003;285:C1036-C46.
- [33] Soares MP, Seldon MP, Gregoire IP, Vassilevskaia T, Berberat PO, Yu J, et al. Heme oxygenase-1 modulates the expression of adhesion molecules associated with endothelial cell activation. *J Immunol* 2004;172:3553-63.
- [34] Wu BJ, Midwinter RG, Cassano C, Beck K, Wang Y, Changsiri D, et al. Heme oxygenase-1 increases endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 2009;29:1537-42.
- [35] Wijayanti N, Katz N, Immenschuh S. Biology of heme in health and disease. *Curr Med Chem* 2004;11:981-6.
- [36] Mense SM, Zhang L. Heme: a versatile signaling molecule controlling the activities of diverse regulators ranging from transcription factors to MAP kinases. *Cell Res* 2006;16:681-92.
- [37] Ryter SW, Tyrrell RM. The heme synthesis and degradation pathways: role in oxidant sensitivity. Heme oxygenase has both pro- and antioxidant properties. *Free Radical Biol Med* 2000;28:289-309.
- [38] Ponka P. Cell biology of heme. *Am J Med Sci* 1999;318:241-56.
- [39] Wagener FA, Volk HD, Willis D, Abraham NG, Soares MP, Adema GJ, et al. Different faces of the heme-heme oxygenase system in inflammation. *Pharmacol Rev* 2003;55:551-71.
- [40] Wagener FA, Abraham NG, van Kooyk Y, de Witte T, Figdor CG. Heme-induced cell adhesion in the pathogenesis of sickle-cell disease and inflammation. *Trends Pharmacol Sci* 2001;22:52-4.
- [41] Pamplona A, Ferreira A, Balla J, Jeney V, Balla G, Epiphanyo S, et al. Heme oxygenase-1 and carbon monoxide suppress the pathogenesis of experimental cerebral malaria. *Nat Med* 2007;13:703-10.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [42] Stocker R. Antioxidant activities of bile pigments. *Antioxid Redox Signal* 2004;6:841-9.
- [43] Kapitulnik J, Maines MD. Pleiotropic functions of biliverdin reductase: cellular signaling and generation of cytoprotective and cytotoxic bilirubin. *Trends Pharmacol Sci* 2009;30:129-37.
- [44] Berberat PO, A-Rahim YI, Yamashita K, Warny MM, Csizmadia E, Robson SC, et al. Heme oxygenase-1-generated biliverdin ameliorates experimental murine colitis. *Inflamm Bowel Dis* 2005;11:350-9.
- [45] Kawamura K, Ishikawa K, Wada Y, Kimura S, Matsumoto H, Kohro T, et al. Bilirubin from heme oxygenase-1 attenuates vascular endothelial activation and dysfunction. *Arterioscler Thromb Vasc Biol* 2005;25:155-60.
- [46] Keshavan P, Deem TL, Schwemberger SJ, Babcock GF, Cook-Mills JM, Zucker SD. Unconjugated bilirubin inhibits VCAM-1-mediated transendothelial leukocyte migration. *J Immunol* 2005;174:3709-18.
- [47] Boczkowski J, Poderoso JJ, Motterlini R. CO-metal interaction: Vital signaling from a lethal gas. *Trends Biochem Sci* 2006;31:614-21.
- [48] Kim HP, Ryter SW, Choi AM. CO as a cellular signaling molecule. *Annu Rev Pharmacol Toxicol* 2006;46:411-49.
- [49] Motterlini R, Clark JE, Foresti R, Sarathchandra P, Mann BE, Green CJ. Carbon monoxide-releasing molecules: characterization of biochemical and vascular activities. *Circ Res* 2002;90:E17-24.
- [50] Motterlini R, Mann BE, Foresti R. Therapeutic applications of carbon monoxide-releasing molecules. *Expert Opin Investig Drugs* 2005;14:1305-18.
- [51] Alam J, Cook JL. How many transcription factors does it take to turn on the heme oxygenase-1 gene? *Am J Respir Cell Mol Biol* 2007;36:166-74.
- [52] Alam A. Multiple elements within the 5' distal enhancer of the mouse heme oxygenase-1 gene mediate induction by heavy metals. *J Biol Chem* 1994; 269:25049-56.
- [53] Alam J, Camhi S, Choi AMK. Identification of a second region upstream of the mouse heme oxygenase-1 gene that functions as a basal level and inducer-dependent transcription enhancer. *J Biol Chem* 1995;270:11977-84.
- [54] Nguyen T, Sherratt PJ, Pickett CB. Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annu Rev Pharmacol Toxicol* 2003;43:233-60.
- [55] Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol* 2007;47:89-116.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [56] Yamada N, Yamaya M, Okinaga S, Nakayama K, Sekizawa K, Shibahara S, et al. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am J Hum Genet* 2000;66:187-95.
- [57] Exner M, Minar E, Wagner O, Schillinger M. The role of heme oxygenase-1 promoter polymorphisms in human disease. *Free Radic Biol Med* 2004;37:1097-104.
- [58] Alam J, Stewart D, Touchard C, Boinapally S, Choi AM, Cook JL. Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene. *J Biol Chem* 1999;274:26071-8.
- [59] Moi P, Chan K, Asunis I, Cao A, Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proc Natl Acad Sci U S A* 1994;91:9926-30.
- [60] Motohashi H, O'Connor T, Katsuoka F, Engel JD, Yamamoto M. Integration and diversity of the regulatory network composed of Maf and CNC families of transcription factors. *Gene* 2002;294:1-12.
- [61] Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, et al. Keap1 represses nuclear activation of antioxidant response elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* 1999;13:76-86.
- [62] Kobayashi M, Yamamoto M. Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. *Antioxid Redox Signal* 2005;7:385-94.
- [63] Kaspar JW, Niture SK, Jaiswal AK. Nrf2:INrf2 (Keap1) signaling in oxidative stress. *Free Radic Biol Med* 2009;47:1304-9.
- [64] Kimura M, Yamamoto T, Zhang J, Itoh K, Kyo M, Kamiya T, et al. Molecular basis distinguishing the DNA binding profile of Nrf2-Maf heterodimer from that of Maf homodimer. *J Biol Chem* 2007;282:33681-90.
- [65] Kobayashi M, Li L, Iwamoto N, Nakajima-Takagi Y, Kaneko H, Nakayama Y, et al. The antioxidant defense system Keap1-Nrf2 comprises a multiple sensing mechanism for responding to a wide range of chemical compounds. *Mol Cell Biol* 2009;29:493-502.
- [66] Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, et al. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat Cell Biol* 2010;12:213-23.
- [67] Zhang J, Ohta T, Maruyama A, Hosoya T, Nishikawa K, Maher JM, et al. BRG1 interacts with Nrf2 to selectively mediate HO-1 induction in response to oxidative stress. *Mol Cell Biol* 2006;26:7942-52.
- [68] Oyake T, Itoh K, Motohashi H, Hayashi N, Hoshino H, Nishizawa M, et al. Bach proteins belong to a novel family of BTB-basic leucine zipper transcription factors that

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- interact with MafK and regulate transcription through the NF-E2 site. *Mol Cell Biol* 1996;16:6083-95.
- [69] Igarashi K, Sun J. The heme-Bach1 pathway in the regulation of oxidative stress response and erythroid differentiation. *Antioxid Redox Signal* 2006;8:107-18.
- [70] Ogawa K, Sun J, Taketani S, Nakajima O, Nishitani C, Sassa S, et al. Heme mediates derepression of Maf recognition element through direct binding to transcription repressor Bach1. *Embo J* 2001;20:2835-43.
- [71] Zenke-Kawasaki Y, Dohi Y, Katoh Y, Ikura T, Ikura M, Asahara T, et al. Heme induces ubiquitination and degradation of the transcription factor Bach1. *Mol Cell Biol* 2007;27:6962-71.
- [72] Tanimoto T, Hattori N, Senoo T, Furonaka M, Ishikawa N, Fujitaka K, et al. Genetic ablation of the Bach1 gene reduces hyperoxic lung injury in mice: role of IL-6. *Free Radic Biol Med* 2009;46:1119-26.
- [73] Suzuki H, Tashiro S, Sun J, Doi H, Satomi S, Igarashi K. Cadmium induces nuclear export of Bach1, a transcriptional repressor of heme oxygenase-1 gene. *J Biol Chem* 2003;278:49246-53.
- [74] Ishikawa M, Numazawa S, Yoshida T. Redox regulation of the transcriptional repressor Bach1. *Free Radic Biol Med* 2005;38:1344-52.
- [75] Reichard JF, Sartor MA, Puga A. BACH1 is a specific repressor of HMOX1 that is inactivated by arsenite. *J Biol Chem* 2008;283:22363-70.
- [76] Okada S, Muto A, Ogawa E, Nakanome A, Katoh Y, Ikawa S, et al. Bach1-dependent and -independent regulation of heme oxygenase-1 in keratinocytes. *J Biol Chem* 2010.
- [77] MacLeod AK, McMahon M, Plummer SM, Higgins LG, Penning TM, Igarashi K, et al. Characterization of the cancer chemopreventive NRF2-dependent gene battery in human keratinocytes: demonstration that the KEAP1-NRF2 pathway, and not the BACH1-NRF2 pathway, controls cytoprotection against electrophiles as well as redox-cycling compounds. *Carcinogenesis* 2009;30:1571-80.
- [78] Hintze KJ, Katoh Y, Igarashi K, Theil EC. Bach1 repression of ferritin and thioredoxin reductase1 is heme-sensitive in cells and in vitro and coordinates expression with heme oxygenase1, beta-globin, and NADP(H) quinone (oxido) reductase1. *J Biol Chem* 2007;282:34365-71.
- [79] Dhakshinamoorthy S, Jain AK, Bloom DA, Jaiswal AK. Bach1 competes with Nrf2 leading to negative regulation of the antioxidant response element (ARE)-mediated NAD(P)H:quinone oxidoreductase 1 gene expression and induction in response to antioxidants. *J Biol Chem* 2005;280:16891-900.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [80] Perkins ND, Gilmore TD. Good cop, bad cop: the different faces of NF-kappaB. *Cell Death Differ* 2006;13:759-72.
- [81] Gilmore TD. Introduction to NF-kappaB: players, pathways, perspectives. *Oncogene* 2006;25:6680-4.
- [82] Ghosh S, Hayden MS. New regulators of NF-kappaB in inflammation. *Nat Rev Immunol* 2008;8:837-48.
- [83] Chen LF, Greene WC. Shaping the nuclear action of NF-kappaB. *Nat Rev Mol Cell Biol* 2004;5:392-401.
- [84] Naidu S, Wijayanti N, Santoso S, Kietzmann T, Immenschuh S. An atypical NF-kappa B-regulated pathway mediates phorbol ester-dependent heme oxygenase-1 gene activation in monocytes. *J Immunol* 2008;181:4113-23.
- [85] Li Q, Guo Y, Ou Q, Cui C, Wu WJ, Tan W, et al. Gene transfer of inducible nitric oxide synthase affords cardioprotection by upregulating heme oxygenase-1 via a nuclear factor- κ B-dependent pathway. *Circulation* 2009;120:1222-30.
- [86] Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol Chem* 1995;270:16483-6.
- [87] Hess J, Angel P, Schorpp-Kistner M. AP-1 subunits: quarrel and harmony among siblings. *J Cell Sci* 2004;117:5965-73.
- [88] Alam J, Den Z. Distal AP-1 binding sites mediate basal level enhancement and TPA induction of the mouse heme oxygenase-1 gene. *J Biol Chem* 1992;267:21894-900.
- [89] Immenschuh S, Hinke V, Ohlmann A, Gifhorn-Katz S, Katz N, Jungermann K, et al. Transcriptional activation of the haem oxygenase-1 gene by cGMP via a cAMP response element/activator protein-1 element in primary cultures of rat hepatocytes. *Biochem J* 1998;334 (Pt 1):141-6.
- [90] Hock TD, Liby K, Wright MM, McConnell S, Schorpp-Kistner M, Ryan TM, et al. JunB and JunD regulate human heme oxygenase-1 gene expression in renal epithelial cells. *J Biol Chem* 2007;282:6875-86.
- [91] Immenschuh S, Hinke V, Katz N, Kietzmann T. Transcriptional induction of the heme oxygenase-1 gene expression by okadaic acid in primary rat hepatocyte cultures. *Mol Pharmacol* 2000;57:610-8.
- [92] Levy S, Jaiswal AK, Forman HJ. The role of c-Jun phosphorylation in EpRE activation of phase II genes. *Free Radic Biol Med* 2009;47:1172-9.
- [93] Yang H, Magilnick N, Lee C, Kalmaz D, Ou X, Chan JY, et al. Nrf1 and Nrf2 regulate rat glutamate-cysteine ligase catalytic subunit transcription indirectly via NF-kappaB and AP-1. *Mol Cell Biol* 2005;25:5933-46.
- [94] Wijayanti N, Kietzmann T, Immenschuh S. Heme oxygenase-1 gene activation by the NAD(P)H oxidase inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride via a protein

- kinase B, p38-dependent signaling pathway in monocytes. *J Biol Chem* 2005;280:21820-9.
- [95] Samoylenko A, Dimova E, Teplyuk N, Immenschuh S, Kietzmann T. Opposite expression of the antioxidant heme oxygenase-1 in primary cells and tumor cells: regulation by interaction of USF-2 and Fra-1. *Antioxid Redox Signal* 2008;in press.
- [96] Wright MM, Kim J, Hock TD, Leitinger N, Freeman BA, Agarwal A. Human haem oxygenase-1 induction by nitro-linoleic acid is mediated by cAMP, AP-1 and E-box response element interactions. *Biochem J* 2009;422:353-61.
- [97] Deshane J, Kim J, Bolisetty S, Hock TD, Hill-Kapturczak N, Agarwal A. Sp1 regulates chromatin looping between an intronic enhancer and distal promoter of the human heme oxygenase-1 gene in renal cells. *J Biol Chem* 2010;285:16476-86.
- [98] Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* 2001;81:807-69.
- [99] Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. *Nat Rev Cancer* 2009;9:537-49.
- [100] Kietzmann T, Samoylenko A, Immenschuh S. Transcriptional regulation of heme oxygenase-1 gene expression by MAP kinases of the JNK and p38 pathways in primary cultures of rat hepatocytes. *J Biol Chem* 2003;278:17927-36.
- [101] Wijayanti N, Huber S, Samoylenko A, Kietzmann T, Immenschuh S. Role of NF-kappaB and p38 MAP kinase signaling pathways in the lipopolysaccharide-dependent activation of heme oxygenase-1 gene expression. *Antioxid Redox Signal* 2004;6:802-10.
- [102] Yu R, Mandlekar S, Lei W, Fahl WE, Tan TH, Kong AN. p38 mitogen-activated protein kinase negatively regulates the induction of phase II drug-metabolizing enzymes that detoxify carcinogens. *J Biol Chem* 2000;275:2322-7.
- [103] Keum YS, Yu S, Chang PP, Yuan X, Kim JH, Xu C, et al. Mechanism of action of sulforaphane: inhibition of p38 mitogen-activated protein kinase isoforms contributing to the induction of antioxidant response element-mediated heme oxygenase-1 in human hepatoma HepG2 cells. *Cancer Res* 2006;66:8804-13.
- [104] Naidu S, Vijayan V, Santoso S, Kietzmann T, Immenschuh S. Inhibition and genetic deficiency of p38 MAPK up-regulates heme oxygenase-1 gene expression via Nrf2. *J Immunol* 2009;182:7048-57.
- [105] Dolado I, Swat A, Ajenjo N, De Vita G, Cuadrado A, Nebreda AR. p38alpha MAP kinase as a sensor of reactive oxygen species in tumorigenesis. *Cancer Cell* 2007;11:191-205.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [106] Silva G, Cunha A, Gregoire IP, Seldon MP, Soares MP. The antiapoptotic effect of heme oxygenase-1 in endothelial cells involves the degradation of p38 alpha MAPK isoform. *J Immunol* 2006;177:1894-903.
- [107] Koyasu S. The role of PI3K in immune cells. *Nat Immunol* 2003;4:313-9.
- [108] Ghigo A, Damilano F, Braccini L, Hirsch E. PI3K inhibition in inflammation: Toward tailored therapies for specific diseases. *Bioessays* 2010;32:185-96.
- [109] Martin D, Rojo AI, Salinas M, Diaz R, Gallardo G, Alam J, et al. Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical carnosol. *J Biol Chem* 2004;279:8919-29.
- [110] Alvarez-Maqueda M, El Bekay R, Alba G, Monteseirin J, Chacon P, Vega A, et al. 15-deoxy-delta 12,14-prostaglandin J2 induces heme oxygenase-1 gene expression in a reactive oxygen species-dependent manner in human lymphocytes. *J Biol Chem* 2004;279:21929-37.
- [111] Hamdulay SS, Wang B, Birdsey GM, Ali F, Dumont O, Evans PC, et al. Celecoxib activates PI-3K/Akt and mitochondrial redox signaling to enhance heme oxygenase-1-mediated anti-inflammatory activity in vascular endothelium. *Free Radic Biol Med* 2010;48:1013-23.
- [112] Salazar M, Rojo AI, Velasco D, de Sagarra RM, Cuadrado A. Glycogen synthase kinase-3beta inhibits the xenobiotic and antioxidant cell response by direct phosphorylation and nuclear exclusion of the transcription factor Nrf2. *J Biol Chem* 2006;281:14841-51.
- [113] Kaspar JW, Jaiswal AK. Antioxidant-induced phosphorylation of tyrosine 486 leads to rapid nuclear export of Bach1 that allows Nrf2 to bind to the antioxidant response element and activate defensive gene expression. *J Biol Chem* 2010;285:153-62.
- [114] Lee TS, Chau LY. Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. *Nat Med* 2002;8:240-6.
- [115] Chen S, Kapturczak MH, Wasserfall C, Glushakova OY, Campbell-Thompson M, Deshane JS, et al. Interleukin 10 attenuates neointimal proliferation and inflammation in aortic allografts by a heme oxygenase-dependent pathway. *Proc Natl Acad Sci U S A* 2005;102:7251-6.
- [116] Ricchetti GA, Williams LM, Foxwell BM. Heme oxygenase 1 expression induced by IL-10 requires STAT-3 and phosphoinositol-3 kinase and is inhibited by lipopolysaccharide. *J Leukoc Biol* 2004;76:719-26.
- [117] Mandal P, Park PH, McMullen MR, Pratt BT, Nagy LE. The anti-inflammatory effects of adiponectin are mediated via a heme oxygenase-1-dependent pathway in rat Kupffer cells. *Hepatology* 2010;51:1420-9.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [118] Zhang X, Shan P, Jiang G, Zhang SS, Otterbein LE, Fu XY, et al. Endothelial STAT3 is essential for the protective effects of HO-1 in oxidant-induced lung injury. *Faseb J* 2006;20:2156-8.
- [119] Tron K, Samoylenko A, Musikowski G, Kobe F, Immenschuh S, Schaper F, et al. Regulation of rat heme oxygenase-1 expression by interleukin-6 via the Jak/STAT pathway in hepatocytes. *J Hepatol* 2006;45:72-80.
- [120] Weis N, Weigert A, von Knethen A, Brune B. Heme oxygenase-1 contributes to an alternative macrophage activation profile induced by apoptotic cell supernatants. *Mol Biol Cell* 2009;20:1280-8.
- [121] Figueiredo RT, Fernandez PL, Mourao-Sa DS, Porto BN, Dutra FF, Alves LS, et al. Characterization of heme as activator of Toll-like receptor 4. *J Biol Chem* 2007;282:20221-9.
- [122] Nakahira K, Kim HP, Geng XH, Nakao A, Wang X, Murase N, et al. Carbon monoxide differentially inhibits TLR signaling pathways by regulating ROS-induced trafficking of TLRs to lipid rafts. *J Exp Med* 2006;203:2377-89.
- [123] Wang XM, Kim HP, Nakahira K, Ryter SW, Choi AM. The heme oxygenase-1/carbon monoxide pathway suppresses TLR4 signaling by regulating the interaction of TLR4 with caveolin-1. *J Immunol* 2009;182:3809-18.
- [124] Lee TS, Chang CC, Zhu Y, Shyy JY. Simvastatin induces heme oxygenase-1: a novel mechanism of vessel protection. *Circulation* 2004;110:1296-302.
- [125] Grosser N, Hemmerle A, Berndt G, Erdmann K, Hinkelmann U, Schurger S, et al. The antioxidant defense protein heme oxygenase 1 is a novel target for statins in endothelial cells. *Free Radic Biol Med* 2004;37:2064-71.
- [126] Horvath K, Varga C, Berko A, Posa A, Laszlo F, Whittle BJ. The involvement of heme oxygenase-1 activity in the therapeutic actions of 5-aminosalicylic acid in rat colitis. *Eur J Pharmacol* 2008;581:315-23.
- [127] Rahman I, Biswas SK, Kirkham PA. Regulation of inflammation and redox signaling by dietary polyphenols. *Biochem Pharmacol* 2006;72:1439-52.
- [128] Kiemer AK, Gerwig T, Gerbes AL, Meissner H, Bilzer M, Vollmar AM. Kupffer-cell specific induction of heme oxygenase 1 (hsp32) by the atrial natriuretic peptide-role of cGMP. *J Hepatol* 2003;38:490-8.
- [129] Loke WM, Proudfoot JM, Hodgson JM, McKinley AJ, Hime N, Magat M, et al. Specific dietary polyphenols attenuate atherosclerosis in apolipoprotein E-knockout mice by alleviating inflammation and endothelial dysfunction. *Arterioscler Thromb Vasc Biol* 2010;30:749-57.

- 1 [130] Paul G, Bataille F, Obermeier F, Bock J, Klebl F, Strauch U, et al. Analysis of
2 intestinal haem-oxygenase-1 (HO-1) in clinical and experimental colitis. Clin Exp
3 Immunol 2005;140:547-55.
4
5 [131] Nakamichi I, Habtezion A, Zhong B, Contag CH, Butcher EC, Omary MB. Hemin-
6 activated macrophages home to the pancreas and protect from acute pancreatitis via
7 heme oxygenase-1 induction. J Clin Invest 2005;115:3007-14.
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

FIGURE LEGENDS

Figure 1: Regulatory role of HO-1 in the inflammatory response of macrophages

Schematic presentation on how HO-1 and its products BR and CO might control the balance of pro- and anti-inflammatory cytokines in macrophages. This balance plays a major role in the activation of macrophages and may thus be critical for the regulation of inflammatory reactions. *Abbreviations:* BR, bilirubin; CO, carbon monoxide; Fe, ferrous iron; IL, interleukin; LPS, lipopolysaccharide; LTA, lipoteichoic acid; TNF α , tumor necrosis factor- α .

Figure 2: Regulation of HO-1 gene expression via the redox-dependent TFs Nrf2 and Bach1

HO-1 gene expression is regulated via the TFs Nrf2 and Bach1, which have counter-regulatory functions. Under basal conditions the transcription repressor Bach1 binds to AREs of the HO-1 promoter. When cellular heme levels are high and in response to stress stimuli, Bach1 is removed from the HO-1 promoter. In addition, stress stimuli cause dissociation of Nrf2 from Keap1, which activates HO-1 gene expression after nuclear translocation via binding to HO-1 AREs. Thus, the interplay between Bach1 and Nrf2 appears to be crucial for the regulation of inducible HO-1 gene expression. *Abbreviations:* ARE, antioxidant response element; Bach1, BTB and CNC homologue 1; HO-1, heme oxygenase-1; Keap1, Kelch-like ECH-associated protein 1; Nrf2, NF-E2-related factor 2; ROS; reactive oxygen species.

Figure 3: Signaling cascades that target Keap1/Nrf2 and Bach1 to regulate HO-1 gene expression

Schematic presentation of major signaling cascades that are involved in the regulation of HO-1 gene expression via interacting with Nrf2 and Bach1. The MAPK p38 α has been shown to activate, but also to inhibit HO-1 gene expression via the TF Nrf2. GSK3 β -mediated phosphorylation has been shown to regulate the activity of Nrf2 and Bach1. *Abbreviations:* ARE, antioxidant response element; Bach1, BTB and CNC homologue 1; ERK, extracellular-

regulated kinase; GSK3 β , glycogen synthase kinase-3 β ; Keap1, Kelch-like ECH-associated
protein 1; Nrf2, NF-E2-related factor 2; PI3-K, phosphatidylinositol-3 kinase.

Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65







