

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Therapeutic Approaches for Targeting Hypoxia-Inducible Factor in Multiple Myeloma

Keita Kirito
University of Yamanashi
Japan

1. Introduction

1.1 Regulation of HIF function

Hypoxia-inducible factor (HIF) is a transcription factor that is a master regulator of cellular responses to hypoxia and regulates many genes that are required for adaptation to hypoxia. ¹HIF is composed of two subunits, HIF α and HIF β . HIF α is composed of three family members, HIF-1 α , HIF-2 α and HIF-3 α . ²Among these family members, HIF-1 α and HIF-2 α play crucial roles in hypoxic responses. HIF activity is regulated via two mechanisms in response to hypoxia. First, HIF α expression is dependent on oxygen levels. ¹ Under normoxic conditions, HIF α is hydroxylated at specific proline residues via prolyl-hydroxylase domain proteins (PHDs). The enzymatic activities of PHDs are dependent on oxygen levels. Hydroxylation of the proline residues of HIFs increases the interaction of HIF α with the von Hippel-Lindau (VHL) proteins, which recruit an E3 ubiquitin-protein ligase. In turn, HIF α is ubiquitinated and subsequently degraded by the proteasome. In hypoxic conditions, PHD activity is suppressed and HIF α degradation is reduced. In addition to protein levels, oxygen levels control the transcriptional activities of HIFs. ²The factors that inhibit HIF (FIHs) play central roles in the process. At normoxic conditions, FIHs catalyze hydroxylation of asparagine residues of HIF α , which represses the interaction of HIF with the transcriptional coactivator CBP/P300. Using these mechanisms, HIF is activated during hypoxia under normal physiological conditions.

2. Abnormal activation of HIFs in multiple myeloma

Under physiological conditions, HIFs are active during hypoxia. However, HIFs can be activated in many types of cancer cells, even under normoxic conditions, by oncogene products, impaired activities of tumor suppressor genes,³ or the accumulation of metabolic glucose intermediates. ⁴ HIFs are known to be activated in multiple myeloma cells. The hypoxic microenvironment of multiple myeloma bone marrow and oxygen-independent mechanisms contribute to abnormal activation of HIFs in myeloma cells. Asosingh and colleagues reported HIF-1 α expression in 5T2 MM cells in the mouse myeloma model. ⁵These researchers showed that the hypoxic microenvironment of the bone marrow contributed to HIF-1 activation, especially in the initial stages of the disease. ⁵ Hypoxia-induced HIF-1 α expression in myeloma cells in multiple myeloma bone marrow was

reported by Hu et al, who used the murine 5T3MM myeloma model to demonstrate that the majority of myeloma cells localize in an extensively hypoxic region and show strong expression of HIF-1 α .⁶ The correlation between bone marrow hypoxia and HIF-1 activation in multiple myeloma cells was detected in human clinical samples.⁷ Colla and colleagues analyzed the expression of HIF-1 α in bone marrow biopsies and demonstrated HIF-1 α immunostaining in the nuclei of myeloma cells in all multiple myeloma patients.⁷ Additionally, Colla and colleagues analyzed oxygen levels of bone marrow samples from multiple myeloma patients and found that the bone marrow of the myeloma patients was hypoxic. The researchers speculated that this hypoxia may cause the accumulation of HIF-1 α . However, the oxygen levels in the bone marrow of healthy volunteers were equivalent to those of myeloma patients. Therefore, it is not clear whether a reduced oxygen level in the bone marrow is the only inducer of HIF-1 α expression. Colla and colleagues detected HIF-1 α protein in isolated CD138-positive myeloma cells in approximately 28% of myeloma patients in normoxic conditions.⁷ These observations suggest that the hypoxia-independent mechanisms regulate HIF-1 α in multiple myeloma cells. Our group detected constitutive expression of HIF-1 α in several multiple myeloma cell lines and in CD138-positive primary myeloma cells even in normoxic conditions.⁸ We found that growth factors for myeloma cells, including insulin-like growth factor-1 (IGF-1) and IL-6, enhanced the expression of HIF-1 α in myeloma cells through activation of AKT. LY294002, which is an inhibitor of PI3-kinase and AKT, inhibited IGF-1-induced HIF-1 α elevation.⁸ The oncogene product c-Myc is also involved in aberrant activation of HIF-1 under normoxia.⁹ ¹⁰Downregulation of c-Myc by chemical inhibitors or siRNA diminished HIF-1 α expression levels in myeloma cells.⁹ Increased DNA methylation was detected in the promoter region of VHL¹¹ and PHD¹² genes in myeloma cells and may contribute to abnormal HIF activation. In addition to protein levels, transcriptional activity of HIFs is modulated in multiple myeloma cells. The inhibitor of growth family member 4 (ING4) is a tumor suppressor gene that is involved in the process. Colla et al. found that myeloma cells showed reduced ING4 expression, which inversely correlated with the expression of pro-angiogenic factors such as IL-8 and osteopontin.¹³ Colla et al. concluded that ING4 suppressed HIF-1 function via PHD interactions.¹³

Abnormal activation of HIF-2 is found in multiple myeloma cells. Martin et al. examined the expression of HIF-1 α and HIF-2 α with bone marrow trephine specimens from patients with multiple myeloma.¹⁴ They detected weak HIF-1 α expression in numerous types of bone marrow cells, whereas HIF-2 α expression was strong and restricted to CD138-positive cells.¹⁴ Abnormal activation of HIF-2 was reported by another group.¹⁵ In their study, the expression of HIF-1 α and HIF-2 α was assessed in the bone marrow of 106 multiple myeloma patients. Among the patients, HIF-1 α and HIF-2 α were expressed in 33% and 13.2%, respectively.¹⁵ The aforementioned findings indicate that HIF-1 α and HIF-2 are activated in multiple myeloma cells using multiple mechanisms, including the hypoxic environment of the myeloma bone marrow, growth factors for myeloma cells and oncogene products (Fig.1).

3. Role of abnormal HIF activation in the pathophysiology of MM

HIF α forms a complex with the beta-subunit of HIF and binds conserved DNA sequences that are known as hypoxia-response elements (5'-RCGTG-3') to regulate mRNA expression of target genes.¹ HIFs regulate many genes that are required for adaptation to hypoxic conditions. Among the first group of genes regulated by HIF are pro-angiogenic factors

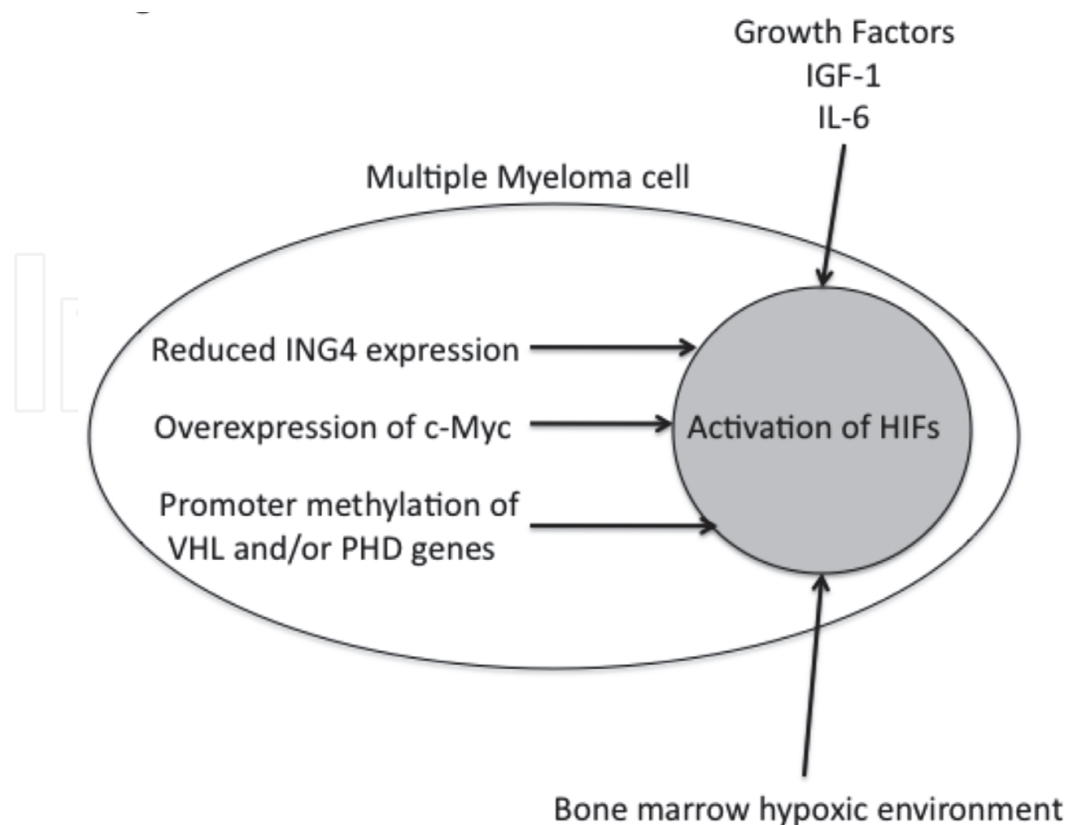


Fig. 1. Abnormal activation of HIFs in multiple myeloma cells

The bone marrow of multiple myeloma patients is hypoxic, which induces HIF activation in multiple myeloma cells. Growth factors activate HIFs via increased protein synthesis in myeloma cells. Overexpression of c-Myc and decreased ING4 enhance HIF activity. DNA methylation of the promoter regions of VHL and PDHs, which are required for HIF α degradation, may enhance HIF α levels.

including vascular endothelial growth factor (VEGF), IL-8 and osteopontin.¹³ HIFs regulate several chemokines and their receptors SDF-1¹⁶ (also known as CXCL12) and CXCR4.¹⁷ Several anti-apoptotic proteins are also HIF targets. HIF-1 regulates the Bcl-2 family of anti-apoptotic proteins: Bcl-xL¹⁸ and Mcl-1.¹⁹ Furthermore, HIF-1 controls the expression of survivin, which is a member of the inhibitor for apoptosis family of proteins (IAPs).²⁰ Under hypoxic conditions, glucose metabolism is shifted from oxidative phosphorylation, which requires oxygen, to glycolysis. HIFs contribute to this metabolic shift²¹ via up-regulation of glycolytic enzymes including glucose transporters, hexokinase and pyruvate dehydrogenase kinase (PDK).²²⁻²⁴ In addition, HIFs suppress mitochondrial function to reduce oxidative phosphorylation.²¹

Aberrant HIF activation in multiple myeloma cells enhances the expression of these genes and contributes to the pathophysiology of multiple myeloma. HIFs enhance VEGF production in myeloma cells.⁹ In addition, HIF-1 may contribute to increased angiogenesis via up-regulation of IL-8 and osteopontin.¹³ Osteopontin stimulates the survival and migration of endothelial cells to enhance angiogenesis.²⁵ HIF-2 activation induces CXCL12 expression, which contributes to aberrant angiogenesis.¹⁴ The promoter of CXCL12 has several hypoxia responsive elements (HREs). Treatment of myeloma cell lines with hypoxia

increases the binding of HIF-2 to HRE on the CXCL12 promoter and enhances transcriptional activity. Ectopic expression of HIF-2 in myeloma cells increases production of CXCL2. The role of CXCL12 in angiogenesis was revealed by a study using a mouse xenograft model. In this model, the addition of an agonist for CXCR4, which is a receptor for CXCL12, inhibited angiogenesis.¹⁴ In addition to pathological angiogenesis, HIFs induce survival molecules in multiple myeloma cells. We found that activation of HIF-1 by IGF-1 enhanced the expression of the anti-apoptotic protein survivin.⁸ Recently, target genes of HIFs in multiple myeloma cells were analyzed using high-throughput methods.⁷ Colla et al. compared the transcriptional profile of CD138-positive myeloma cells under normoxia and treated with the hypoxic mimetic drug CoCl₂. They detected 714 genes that were significantly modulated by hypoxia including heme oxygenase-1, heat shock protein 90 (Hsp90), VEGF and IL-8.⁷ In summary, HIFs contribute to the pathogenesis of multiple myeloma by inducing the expression of their target genes to enhance angiogenesis in the bone marrow and suppressing apoptosis in myeloma cells (Fig. 2).

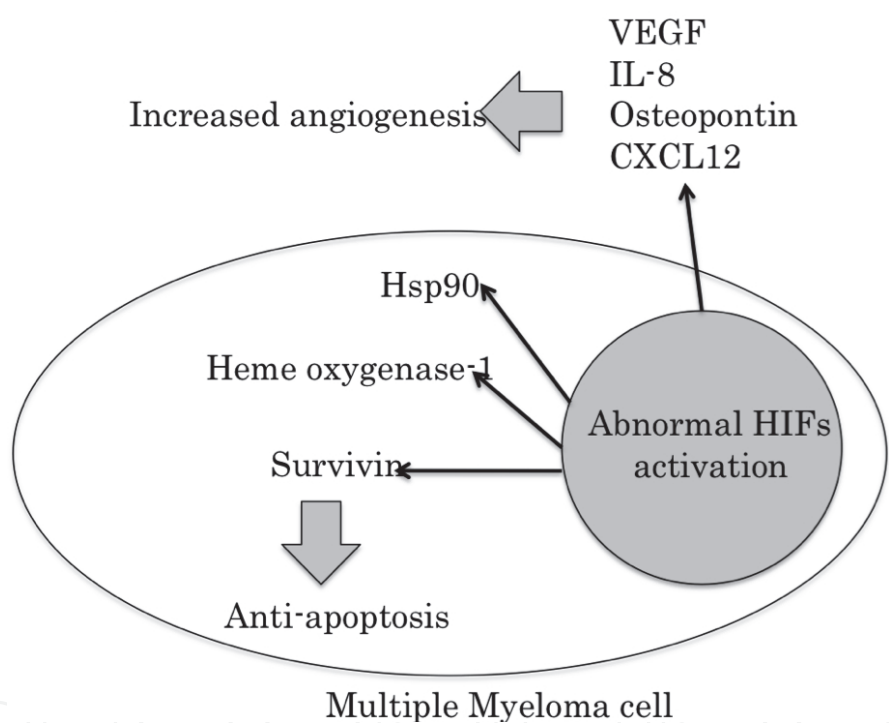


Fig. 2. Pathological role of HIFs in multiple myeloma
HIF activation induces the production of several pro-angiogenetic factors and enhances angiogenesis in the bone marrow. HIFs suppress apoptosis in myeloma cells by inducing anti-apoptotic factors.

4. HIFs as therapeutic targets for multiple myeloma

As described above, evidence has accumulated that implicates the important role of HIFs in the pathophysiology of multiple myeloma. These findings suggest the possibility for new therapeutic approaches that target hypoxic bone marrow microenvironments and HIFs. Our group showed that inhibition of HIF-1 function via the chemical inhibitor echinomycin²⁶, which disrupts HIF binding to DNA, or siRNA against HIF-1 α disrupted the anti-apoptotic effects of IGF-1.⁸ Importantly, echinomycin enhanced melphalan-induced apoptosis in

primary CD138-positive myeloma cells.⁸ Zhang et al. found that adaphostin, which is a tyrosine kinase inhibitor,^{27, 28} blocked expression of c-Myc and HIF-1 α in several multiple myeloma cells.⁹ They reported that adaphostin down-regulated c-Myc and HIF-1 α in the xenograft mouse myeloma model. In this model, the compound suppressed VEGF secretion, tumor angiogenesis and tumor progression and increased the survival of the animals.⁹ Known anti-myeloma drugs inhibit function of HIFs. Bortezomib suppresses transcriptional activity of HIF-1.²⁹ Lenalidomide inhibits the synthesis of HIF-1 α in endothelial cells and suppresses the expression of HIF-1 α in multiple myeloma cells.³⁰ Recent studies also revealed that HIFs were molecular targets of a new generation of agents against multiple myeloma. A molecular chaperone, heat shock protein 90 (Hsp90), stabilizes a series of proteins that are required for cell cycle progression and survival.³¹ In multiple myeloma cells, Hsp90 is known to enhance survival, and several inhibitors for Hsp90 show anti-myeloma effects.³¹ Interestingly, HIF-1 α is a target protein for Hsp90.³² Treatment of myeloma cells with Hsp90 inhibitor blocked expression of HIF-1 α expression.³² Together with these observations, there is evidence that Hsp90 inhibitor may exert anti-myeloma effects through the suppression of HIFs. Histone deacetylase (HDAC) inhibitors are attractive new-generation agents against multiple myeloma.³³ HDAC inhibitors suppress the growth and survival of myeloma cells in vitro.³⁴ Furthermore, several HDAC inhibitors are currently being used in clinical trials.³⁵ Although the main target of HDAC inhibitors is histone deacetylase, the inhibitors modify the function of numerous proteins including HIFs. The HDAC inhibitor induces the degradation of HIF-1 α independent of VHL function.^{36, 37} Taken together, HIFs may be a molecular target of HDAC inhibitors in multiple myeloma. Finally, TH-302, which is a hypoxia-activated prodrug,³⁸ shows significant anti-myeloma effects in the murine myeloma model.⁶ These results suggest that the hypoxic environment of multiple myeloma bone marrow might be an attractive therapeutic target.

5. Conclusions

The microenvironment of bone marrow is hypoxic in multiple myeloma and supports the survival and growth of myeloma cells, especially during the initial stage of the disease. Hypoxia enhances HIF activity and induces the production of pro-angiogenic factors. Subsequently, angiogenesis in the bone marrow is enhanced and supports further growth of myeloma cells.³⁹ Growth factors for myeloma cells and intrinsic cellular changes (i.e., increased c-Myc expression and reduced ING4 levels) modify HIF activity and may contribute to increased angiogenesis. Furthermore, HIFs are survival factors for myeloma cells that induce the production of anti-apoptotic proteins and may be involved in the acquisition of drug-resistance. Treatments targeting both HIFs and hypoxic microenvironments represent novel strategies to improve treatment outcomes for multiple myeloma.

6. References

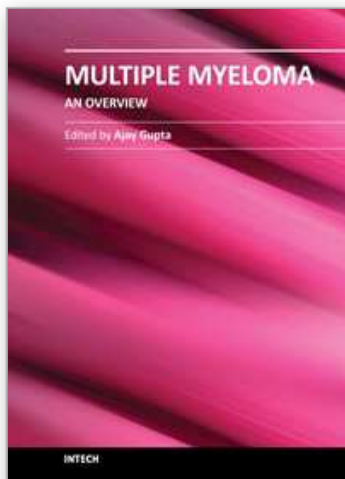
- [1] Semenza GL. Oxygen sensing, homeostasis, and disease. *N Engl J Med*. 2011;365:537-547.
- [2] Lisy K, Peet DJ. Turn me on: regulating HIF transcriptional activity. *Cell Death Differ*. 2008;15:642-649.

- [3] Zundel W, Schindler C, Haas-Kogan D, et al. Loss of PTEN facilitates HIF-1-mediated gene expression. *Genes Dev.* 2000;14:391-396.
- [4] Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene.* 2010;29:625-634.
- [5] Asosingh K, De Raeve H, de Ridder M, et al. Role of the hypoxic bone marrow microenvironment in 5T2MM murine myeloma tumor progression. *Haematologica.* 2005;90:810-817.
- [6] Hu J, Handisides DR, Van Valckenborgh E, et al. Targeting the multiple myeloma hypoxic niche with TH-302, a hypoxia-activated prodrug. *Blood.* 2010;116:1524-1527.
- [7] Colla S, Storti P, Donofrio G, et al. Low bone marrow oxygen tension and hypoxia-inducible factor-1alpha overexpression characterize patients with multiple myeloma: role on the transcriptional and proangiogenic profiles of CD138(+) cells. *Leukemia.* 2010;24:1967-1970.
- [8] Hu Y, Kirito K, Yoshida K, et al. Inhibition of hypoxia-inducible factor-1 function enhances the sensitivity of multiple myeloma cells to melphalan. *Mol Cancer Ther.* 2009;8:2329-2338.
- [9] Zhang J, Sattler M, Tonon G, et al. Targeting angiogenesis via a c-Myc/hypoxia-inducible factor-1alpha-dependent pathway in multiple myeloma. *Cancer Res.* 2009;69:5082-5090.
- [10] Podar K, Anderson KC. A therapeutic role for targeting c-Myc/Hif-1-dependent signaling pathways. *Cell Cycle.* 2010;9:1722-1728.
- [11] Hatzimichael E, Dranitsaris G, Dasoula A, et al. Von Hippel-Lindau methylation status in patients with multiple myeloma: a potential predictive factor for the development of bone disease. *Clin Lymphoma Myeloma.* 2009;9:239-242.
- [12] Hatzimichael E, Dasoula A, Shah R, et al. The prolyl-hydroxylase EGLN3 and not EGLN1 is inactivated by methylation in plasma cell neoplasia. *Eur J Haematol.* 2010;84:47-51.
- [13] Colla S, Tagliaferri S, Morandi F, et al. The new tumor-suppressor gene inhibitor of growth family member 4 (ING4) regulates the production of proangiogenic molecules by myeloma cells and suppresses hypoxia-inducible factor-1 alpha (HIF-1alpha) activity: involvement in myeloma-induced angiogenesis. *Blood.* 2007;110:4464-4475.
- [14] Martin SK, Diamond P, Williams SA, et al. Hypoxia-inducible factor-2 is a novel regulator of aberrant CXCL12 expression in multiple myeloma plasma cells. *Haematologica.* 2010;95:776-784.
- [15] Giatromanolaki A, Bai M, Margaritis D, et al. Hypoxia and activated VEGF/receptor pathway in multiple myeloma. *Anticancer Res.* 2010;30:2831-2836.
- [16] Ceradini DJ, Kulkarni AR, Callaghan MJ, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med.* 2004;10:858-864.
- [17] Staller P, Sulitkova J, Lisztwan J, Moch H, Oakeley EJ, Krek W. Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. *Nature.* 2003;425:307-311.
- [18] Chen N, Chen X, Huang R, et al. BCL-xL Is a Target Gene Regulated by Hypoxia-inducible Factor-1. *J Biol Chem.* 2009;284:10004-10012.

- [19] Piret J-P, Minet E, Cosse J-P, et al. Hypoxia-inducible Factor-1-dependent Overexpression of Myeloid Cell Factor-1 Protects Hypoxic Cells against tert-Butyl Hydroperoxide-induced Apoptosis. *Journal of Biological Chemistry*. 2005;280:9336-9344.
- [20] Peng X-H, Karna P, Cao Z, Jiang B-H, Zhou M, Yang L. Cross-talk between Epidermal Growth Factor Receptor and Hypoxia-inducible Factor-1{alpha} Signal Pathways Increases Resistance to Apoptosis by Up-regulating Survivin Gene Expression. *J Biol Chem*. 2006;281:25903-25914.
- [21] Semenza GL. Hypoxia-inducible factor 1: regulator of mitochondrial metabolism and mediator of ischemic preconditioning. *Biochim Biophys Acta*. 2010;1813:1263-1268.
- [22] Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab*. 2006;3:177-185.
- [23] Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab*. 2006;3:187-197.
- [24] Kirito K, Hu Y, Komatsu N. HIF-1 prevents the overproduction of mitochondrial ROS after cytokine stimulation through induction of PDK-1. *Cell Cycle*. 2009;8:2844-2849.
- [25] Takahashi F, Akutagawa S, Fukumoto H, et al. Osteopontin induces angiogenesis of murine neuroblastoma cells in mice. *Int J Cancer*. 2002;98:707-712.
- [26] Kong D, Park EJ, Stephen AG, et al. Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. *Cancer Res*. 2005;65:9047-9055.
- [27] Avramis IA, Christodouloupoulos G, Suzuki A, et al. In vitro and in vivo evaluations of the tyrosine kinase inhibitor NSC 680410 against human leukemia and glioblastoma cell lines. *Cancer Chemother Pharmacol*. 2002;50:479-489.
- [28] Chandra J, Tracy J, Loegering D, et al. Adaphostin-induced oxidative stress overcomes BCR/ABL mutation-dependent and -independent imatinib resistance. *Blood*. 2006;107:2501-2506.
- [29] Shin DH, Chun YS, Lee DS, Huang LE, Park JW. Bortezomib inhibits tumor adaptation to hypoxia by stimulating the FIH-mediated repression of hypoxia-inducible factor-1. *Blood*. 2008;111:3131-3136.
- [30] Lu L, Payvandi F, Wu L, et al. The anti-cancer drug lenalidomide inhibits angiogenesis and metastasis via multiple inhibitory effects on endothelial cell function in normoxic and hypoxic conditions. *Microvasc Res*. 2009;77:78-86.
- [31] Richardson PG, Mitsiades CS, Laubach JP, Lonial S, Chanan-Khan AA, Anderson KC. Inhibition of heat shock protein 90 (HSP90) as a therapeutic strategy for the treatment of myeloma and other cancers. *Br J Haematol*. 2010;152:367-379.
- [32] Mabeesh NJ, Post DE, Willard MT, et al. Geldanamycin induces degradation of hypoxia-inducible factor 1alpha protein via the proteosome pathway in prostate cancer cells. *Cancer Res*. 2002;62:2478-2482.
- [33] Dimopoulos MA, San-Miguel JF, Anderson KC. Emerging therapies for the treatment of relapsed or refractory multiple myeloma. *Eur J Haematol*. 2011;86:1-15.
- [34] Mandl-Weber S, Meinel FG, Jankowsky R, Oduncu F, Schmidmaier R, Baumann P. The novel inhibitor of histone deacetylase resminostat (RAS2410) inhibits proliferation

- and induces apoptosis in multiple myeloma (MM) cells. *Br J Haematol.* 2010;149:518-528.
- [35] Niesvizky R, Ely S, Mark T, et al. Phase 2 trial of the histone deacetylase inhibitor romidepsin for the treatment of refractory multiple myeloma. *Cancer.* 2011;117:336-342.
- [36] Kong X, Lin Z, Liang D, Fath D, Sang N, Caro J. Histone deacetylase inhibitors induce VHL and ubiquitin-independent proteasomal degradation of hypoxia-inducible factor 1alpha. *Mol Cell Biol.* 2006;26:2019-2028.
- [37] Fath DM, Kong X, Liang D, et al. Histone deacetylase inhibitors repress the transactivation potential of hypoxia-inducible factors independently of direct acetylation of HIF-alpha. *J Biol Chem.* 2006;281:13612-13619.
- [38] Duan JX, Jiao H, Kaizerman J, et al. Potent and highly selective hypoxia-activated achiral phosphoramidate mustards as anticancer drugs. *J Med Chem.* 2008;51:2412-2420.
- [39] Martin SK, Diamond P, Gronthos S, Peet DJ, Zannettino AC. The emerging role of hypoxia, HIF-1 and HIF-2 in multiple myeloma. *Leukemia.* 2011.

IntechOpen



Multiple Myeloma - An Overview

Edited by Dr. Ajay Gupta

ISBN 978-953-307-768-0

Hard cover, 274 pages

Publisher InTech

Published online 20, January, 2012

Published in print edition January, 2012

Multiple myeloma is a malignant disorder characterized by the proliferation of plasma cells. Much insight has been gained into the molecular pathways that lead to myeloma and indeed much more remains to be done. The understanding of these pathways is closely linked to their therapeutic implications and is stressed upon in the initial chapters. Recently, the introduction of newer agents such as bortezomib, lenalidomide, thalidomide, liposomal doxorubicin, etc. has led to a flurry of trials aimed at testing various combinations in order to improve survival. Higher response rates observed with these agents have led to their integration into induction therapies. The role of various new therapies vis a vis transplantation has also been examined. Recent advances in the management of plasmacytomas, renal dysfunction, dentistry as well as mobilization of stem cells in the context of myeloma have also found exclusive mention. Since brevity is the soul of wit our attempt has been to present before the reader a comprehensive yet brief text on this important subject.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Keita Kirito (2012). Therapeutic Approaches for Targeting Hypoxia- Inducible Factor in Multiple Myeloma, Multiple Myeloma - An Overview, Dr. Ajay Gupta (Ed.), ISBN: 978-953-307-768-0, InTech, Available from: <http://www.intechopen.com/books/multiple-myeloma-an-overview/therapeutic-approaches-for-targeting-hypoxia-inducible-factor-in-multiple-myeloma>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen