REVIEW DOI: 10.5504/BBEQ.2011.0102 MB

NOTHING IN EXCESS – LESSONS LEARNED FROM THE EXPRESSION OF HIGH-MOBILITY GROUP PROTEINS TYPE A IN NON-CANCER AND CANCER CELLS

Rumena Petkova[1], Hemanth Tummala[2], Nikolai Zhelev [2]
[1]Scientific Technological Service (STS), Ltd., Sofia, Bulgaria
[2]University of Abertay Dundee, School of Contemporary Sciences, Cancer Systems Biology, Dundee, Scotland, UK
Correspondence to: Nikolai Zhelev
E-mail: n.zhelev@abertay.ac.uk

ABSTRACT

High-mobility group A (HMGA) proteins are major transcription regulators which are abundantly and ubiquitously expressed in undifferentiated cells but present at a low level in somatic cells of adult organisms. Up-regulation of HMGA expression is a frequent finding in cancer, either via direct stimulation of expression by constitutively expressed proto-oncogenic factors such as MYC and JUN or by rearrangements rendering the expression of the HMGA proteins not suppressible by inhibitory factors such as miRNAs. Rearrangements of the HMGA genomic loci resulting in disabling of the control mechanisms of their expression are often seen in tumours of various origin.

A direct relationship between the level of expression of HMGA in mitochondria and the level of accumulation of oxidative damage in cancer cells has been recently noted. On the other hand, mammalian cells deficient in HMGA1 expression are also deficient in utilization of glucose and show the impairment in expression of the insulin receptor and the high levels of oxidative damage of DNA characteristic of diabetes type 2 and the related condition metabolic syndrome. Insulin resistance and metabolic syndrome could be viewed as a premalignant state in which DNA damage is slowly accumulating until the repair machinery of the cell cannot withstand the constant oxidative barrage and surrenders to neoplastic transformation.

Biotechnol. & Biotechnol. Eq. 2011, **25**(4), 2572-2575

Keywords: HMGA, cancer, insulin resistance, metabolic syndrome, oxidative DNA damage

Introduction

What is now proved was once only imagin'd.

William Blake, Proverbs of Hell (circa 1791)

High-Mobility Group (HMG) are a group of chromosomal proteins that assist with transcription, replication, recombination, and DNA repair. The HMG proteins are subdivided into three superfamilies each containing a characteristic functional domain: HMGA – contains an AT-hook domain; HMGB – contains an HMG-box domain and HMGN – contains a nucleosomal binding domain (5, 21, 23, 44).

HMGA (formerly called HMGI/Y/C, now divided into HMGA1 and HMGA2) are nonhistone proteins belonging to a family of regulatory factors that play a major role in maintaining the chromatin architecture and dynamics and the regulation of the expression of numerous genes. HMGA1 and HMGA2 are encoded by two different nuclear genes and the HMGA1 transcript produces three different isoforms via alternative splicing. HMGA proteins typically contain the triple DNA-binding motif (AT-hook) that binds to AT-rich regulatory elements in DNA (34) and an acidic 'tail' at the carboxy terminus. HMGA are considered master regulators of gene expression, though typically not by direct transcriptional activation but, rather, by altering DNA conformation so as to modulate binding of transcription factors onto the AT-rich promoter/enhancer regions of their target genes; or by displacing histone H1, a general transcriptional repressor, from transcription initiation sites (11, 36, 43).

HMGA proteins are abundantly and ubiquitously expressed in embryonic tissues but hardly detectable in normal adult tissues (10, 45). This is only natural as hyperplasticity of chromatin, i.e. prodigious availability for various nuclear transactions (replication, transcription, repair) is inherent to the undifferentiated state of the cell (29). Disturbed regulation – usually, up-regulation – of the expression of the HMGA genes is a common finding in virtually all human cancers, resulting in ectopic expression of proteins characteristic of undifferentiated cells (17, 38). In this sense, the HMGA1 gene may be considered a classical proto-oncogene. HMGA expression may be up-regulated by other transcriptional factors as the promoter regions of the HMGA1 genes in mammals contain conserved binding sites for MYC, AP1 (JUN) and other transcription factors; or it may happen via chromosomal rearrangements in the HMGA genes which render the mRNA non-repressible by tumour suppressor miRNAs such as let-7 (3, 24, 31, 32, 41).

It has been shown that overexpression of HMGA may directly inhibit DNA excision repair of any type, nucleotide excision (NER) or base excision (BER), in the nucleus as well as in mitochondria (1, 2, 15). What is more, the level of expression of HMGA proteins may correlate with the tumour stage and propensity for metastasizing in some types of tumours, constituting a novel prognostic marker for grade of malignancy (20, 22). Overexpression of wild-type or modified, cancer-specific HMGA proteins in cancer, however, seems to be cell type specific (12, 13).

Specificities in the expression of HMGA in normal and cancer cells

In normal cells, HMGA proteins are usually resident to the nucleus up to the S/G2 phase of the cell cycle when a fraction of HMGA1 migrates to the mitochondria and binds to the control element of the mitochondrial DNA known as D-loop (14, 15). This is temporary and in non-cancerous cells the migratory HMGA1 is usually shuttled back to the nucleus by the beginning of M phase. At the time of the relocation of HMGA1 from the nucleus to the cytoplasm, occurs a post-translational phosphorylation of the HMGA1 proteins by CDC2 kinase (35), reducing the capacity of the HMGA1 to bind to DNA by over an order of magnitude, and thus probably facilitating the mobilization of the protein and its shuttling to the cytoplasm in the non-cancerous cell. Also, in nontransformed cells HMGA1 is phosphorylated (i.e. its DNA-binding capacity disabled) upon DNA damage and hyperphosphorylated and subsequently methylated at the early stages of programmed cell death (38), which coincides with the apoptotic condensation of chromatin. In many types of tumour cells, however, HMGA1 is readily identifiable in mitochondria throughout the cell cycle.

Considering the ability of HMGA to suppress DNA repair, this may be a pro-carcinogenic mechanism promoting genetic instability in the transformed cells. Cancer cells are prone to DNA damage for yet another reason, namely, the higher dose of oxidative damage they receive because of their altered metabolism. It has been shown that proper oxidative mitochondrial function is essential for proliferation as well as for differentiation of undifferentiated cells (embryonic stem cells as well as cancer cells), and if it is attenuated for some reason, it interferes with the process of differentiation (26). Undifferentiated cells show a number of traits which distinguish them from differentiated cells in regard to metabolism and general biology. Among these are the high-flux backbone metabolic state, resembling prokaryotic cells in log phase; the low nucleus/cytoplasm ratio; and the lower number of mitochondria per cell. Therefore, undifferentiated cells are typically dependent on anaerobic rather than aerobic means to produce energy. Cancer cells make no exception as they are notoriously ineffective in their utilization of energy sources. They rely mainly on glycolysis as a source of chemical energy, which results in generation of smaller amounts of ATP and higher levels of reactive oxygen species (ROS) than in normal cells (19, 30). The latter is, in turn, probably one of the reasons for the higher level of DNA damage observed in mitochondrial DNA of cancer cells compared to normal cells (7).

Mitochondrial DNA is believed to be particularly sensitive to oxidative agents due to its physical proximity to the site where the reactive oxygen species are formed and to the lack of protective structuring, namely, the absence of histones. Moreover, mitochondria are partially deficient in DNA repair mechanisms, that is, they do not seem to employ the mechanism of nucleotide excision (NER) for the repair of their DNA (40). This could be a matter of evolution-imposed parsimony, as usually the major type of DNA damage in mitochondrial DNA is oxidative, caused by the constant flow of reactive oxygen species generated by oxidative phosphorylation. Therefore, mechanisms for repair of other types of lesions could be deemed redundant and therefore lost in the evolutionary process. It seems that the only exception is the special case of mismatch repair, a particular type of NER endowed with strand specificity, as the DNA polymerases which carry out the final step in any type of DNA repair have an inherent error rate and may incorporate mismatched nucleotides, which, unrepaired, may cause base substitution during the next cycle of replication (39). Recombination is also not an option for repair of mitochondrial DNA. None of the proteins involved in the repair of the mitochondrial genome is coded by its own DNA; rather, the relevant information is transcribed using the nuclear genes, translated in the cytoplasm and the complete repair proteins are subsequently imported in the mitochondrion (8). Therefore, it seems like the natural target for oxidative damage, mitochondrial DNA, is not adequately equipped against oxidative assault. In nontransformed cells, however, the existing mechanisms seem to be capable to arrest or delay oxidative damage accumulation sufficiently well so as for the organism to age at a rate typical for the species. In cancer cells, however, the overexpression of a set of proteins capable of modifying the chromatin architecture, such as HMGA, is likely to result in a further plunge in competence for repair of their DNA compared to normal cells. Quantitative experiments have already demonstrated the relationship between the level of inducible expression of HMGA in mitochondria and the level of mitochondrial dysfunction expressed as accumulation of oxidative damage (27).

The role of oxidative damage in the pathogenesis of insulin resistance

Apart from cancer, there are other human diseases and conditions which have been recently found – quite unexpectedly – to be associated with increased level of oxidative damage of DNA. Experimental proof has been found so far for the association of the risk for development of metabolic syndrome (also known as X-syndrome, a very common and potentially dangerous condition) with certain deficiencies of the mechanism for repair of oxidised bases. Metabolic syndrome, as defined by the set of criteria of the International Diabetes Federation (2006), includes abdominal obesity, high triglyceride level, low levels of high density lipoprotein cholesterol, arterial blood pressure above 140/90 and hyperglycemia and/or insulin resistance, the latter being a unifying criterion for all current definitions of metabolic syndrome. The prevalence of the condition varies between ethnic groups but is estimated to have an average prevalence of 20-25% in the developed countries, rising to the striking 40% after the age of 50 (33). Presence of metabolic syndrome constitutes a major risk factor for diabetes mellitus type 2 as well as for cardiovascular and cerebrovascular disease.

A linkage has been recently found between the risk for developing metabolic syndrome and the Fpg/Nei family of DNA glycosylases. The latter are bifunctional glycosylase/lyases which excise oxidised purines from DNA as well as thymine glycol (an exemplary product of oxidation caused by reactive oxygen species) and create a single-strand break at the resulting abasic site (4). It has already been demonstrated in mouse models that the deficiency of one of the mammalian homologues of Fpg/Nei – Neil1, results in hyperglycemia/obesity/kidney damage phenotype resembling closely the phenotype of human metabolic syndrome (42). Reportedly, mitochondrial DNA from Neil1-deficient mice showed significant levels of DNA damage compared to mitochondrial DNA of control animals. Earlier, Foti et al. (18) described a similar hyperglycemic/obese phenotype in mice deficient in the Hmga1 protein and hypothesized that a similar mechanism operated for human diabetes type 2. Later it was demonstrated that human variants of NEIL1 with decreased enzyme activity may, too, result in increased risk for metabolic syndrome in man (37). Both findings shared a common feature, namely, an unusually high level of oxidative damage found in the DNA of the affected cells.

Increased levels of oxidative stress have been identified as a hallmark of insulin-resistant diabetes and atherosclerosis for decades now (6), but whether it was the DNA damage that induced and promoted the pathological process or was it a product of the impact of other factors that brought the insulin resistance about it was not clear. Over 20 variants of the human HMGA1 gene have been identified so far, with at least four of them resulting in severe insulin resistance and as of now, it seems very likely that variants in the HMGA1 gene are directly responsible for at least a proportion of the cases of diabetes type 2 (9). These diabetes type 2-linked HMGA1 variants affect the coding portions of the gene as well as the untranslated region, which indicates that the preservation of the wildtype protein sequence and the regulation of its expression are equally important. The study in question (9) has not identified homozygous carriers of the 'high-risk' variants, which may mean that carriership of two defective copies may dramatically decrease the fitness of the carrier individuals, causing them to die prenatally or not live long enough to reproduce. Actually, previous observations made on mice carrying two copies of a null *Hmga* allele (18) support this notion. Taken together with the habitually high level of oxidative DNA damage seen in insulin resistant cells, recently, a hypothesis has been proposed placing faulty management of DNA damage in charge of the etiopathogenesis of insulin resistance and atherosclerosis (25, 28) but still answers remain elusive as to the exact induction mechanism.

Conclusion – the excess of courtesy is discourtesy, or why X-syndrome is not just a fashionable term

HMGA proteins are capable of reorganizing architecture of the chromatin, giving access to major regulatory elements and recruiting and organizing transcription factors. Unlike most DNA-binding proteins, HMGA proteins bind to chromatin independently of the underlying DNA sequence, rather, their functional specificity depends on interactions with regulatory factors and/or on their ability to target particular chromatin conformations. This ensures the versatility, and, at the same time, the specificity of the interaction between HMGA and its target DNA domains. Taken together with its direct dependence on potent proliferation factors such as MYC and JUN and their suppressive activity over DNA repair, HMGA1 seems to make an exceptionally good up-regulation target in carcinogenesis and is indeed overexpressed in the majority of cancers. The HMGA proteins are both targets and modulators of differentiating agents, such as retinoic acid. Targeted disruption of the HMGA-DNA binding has already been proposed as an anticancer strategy (16, 24). On the other hand, in non-cancerous cells, if the expression level of HMGA1 is low, the associated oxidative damage would not only produce the metabolic phenotype of impaired glucose tolerance but could, in time, result in just enough unrepaired DNA damage so as to launch an oncogenic transformation.

All in all, it seems that both too little and too much of HMGA do not do any good to a cell, be it a normal or a tumour cell, as the level of oxidative damage increases either way. It could be hypothesized that inherently low levels of HMGA (e.g. resulting from polymorphisms in the respective gene/s) may, via different mechanisms, bring about decreased efficiency of protection of the DNA of the cell against oxidative damage and, respectively, accumulation of potentially carcinogenic oxidative lesions. Once the cell is transformed, it may further deploy the mechanism characteristic of cancer cells so as to increase the level of genomic rearrangements, resulting in constitutive overexpression of HMGA and subsequent unleashing of malignant growth. In this light, insulin resistance and metabolic syndrome could be viewed as a long-term, low-grade premalignant state in which DNA damage is slowly but irrevocably accumulating until the repair machinery of the cell cannot withstand the constant oxidative barrage and surrenders to neoplastic transformation. Certainly, the underlying mechanism is not that simple, as living cells possess additional intricate machinery keeping the integrity of their DNA in check and/or sacrifice irreversibly damaged cells. It is likely, however, that HMGA1 is among the most important participants in the process of managing the fine balance between normal and pathological metabolic state, which becomes even finer and more difficult to manage as the organism ages. Since the prevalence of both cancer and insulin resistance increase with age, it is hard to differentiate the impact of the one or the other, but it could reflect a chain of probabilities and events that are causally linked but its determining factors are too strictly regu lated in the normal cells so that the resulting effects only show with advancement of the aging process.

Acknowledgements

This research was supported by grant No. DO02-69 of the Ministry of Education, Youth and Science of the Republic of Bulgaria.

REFERENCES

1. **Adair J.E., Kwon Y., Dement G.A., Smerdon M.J., Reeves R.** (2005) J. Biol. Chem., **80**, 32184-32192.
2. **Adair J.E., Maloney S.C., Dement G.A., Wertzler K.J., Smerdon M.J., Reeves R.** (2007) Cancer Res., **67**, 6044-6052.
3. **Angelova A., Chakarov St., Tencheva Z.** (1998). Compt. Rend. Acad. Bulg. Sci. 51(5/6), 91-92.
4. **Bandaru V., Sunkara S., Wallace S.S., Bond J.P.** (2002) DNA Repair (Amst.), **1**, 517-529.
5. **Barratt M.J., Hazzalin C.A., Zhelev N.Zh., Mahadevan L.C.** (1994) EMBO J., **13**(19), 4524-4535.
6. **Baynes J.W.** (1991) Diabetes, **40**, 405-412.
7. **Berneburg M., Kamenisch Y., Krutmann J.** (2006) Photochem. Photobiol. Sci. **5**, 190-198.
8. **Boesch P., Weber-Lotfi F., Ibrahim N., Tarasenko V., Cosset A., Paulus F., Lightowlers R.N., Dietrich A.** (2011) Biochim. Biophys. Acta, **1813**, 186-200.
9. **Chiefari E., Tanyolaç S., Paonessa F., Pullinger C.R., Capula C., Iiritano S., Mazza T., Forlin M., Fusco A., Durlach V., Durlach A., Malloy M.J., Kane J.P., Heiner S.W., Filocamo M., Foti D.P., Goldfine I.D., Brunetti A.** (2011) J.A.M.A., **305**, 903-912.
10. **Chakarov S., Chakalova L., Tencheva Z., Angelova A.** (1995) Compt. Rend. Acad. Bulg. Sci., 48 (11-12), 139-141.
11. **Chakarov S., Vassilev P., Tencheva Z., Angelova A.** (1997) Biotechnol. Biotech. Eq., 11(3-4), 46-50.
12. **Chakarov S., Chakalova L., Tencheva Z., Ganev V., Angelova A.** (2000) Life Sci., **66**, 1725-1731.
13. **Crombez K.R., Vanoirbeek E.M., Van de Ven W.J., Petit M.M.** (2005) Mol. Cancer. Res., **3**, 63-70.
14. **Dement G.A., Treff N.R., Magnuson N.S., Franceschi V., Reeves R.** (2005) Exp. Cell Res., **307**, 388-401.
15. **Dement G.A., Maloney S.C., Reeves R.** (2007) Exp. Cell Res., **313**, 77-87.
16. **Di Cello F., Hillion J., Kowalski J., Ronnett B.M., Aderinto A., Huso D.L,. Resar L.M.S.** (2008) Mol. Cancer Ther., **7**, 2090-2095.
17. **Fedele M. and Fusco A.** (2010) Biochim. Biophys. Acta, **1799**, 48-54.
18. **Foti D., Chiefari E., Fedele M., Iuliano R., Brunetti L., Paonessa F., Manfioletti G., Barbetti F., Brunetti A., Croce C.M., Fusco A., Brunetti A.** (2005) Nat. Med., **11**, 765-773.
19. **Frisard M. and Ravussin E.** (2006) Endocrine, **29**, 27-32.
20. **Giannini G., Kim C.J., Di Marcotullio L., Manfioletti G., Cardinali B., Cerignoli F., Ristori E., Zani M., Frati L., Screpanti I., Guilino A.** (2000) Br. J. Cancer, **83**, 1503-1509.
21. **Hock R, Furusawa T, Ueda T, Bustin M.** (2007) Trends Cell Biol., **17**(2), 72-79.
22. **Huang M.L., Chen C.C., Chang L.C.** (2009) Int. J. Colorectal Dis., **24**, 1281-1286.
23. **Kardalinou E., Zhelev N.Zh., Hazzalin C.A., Mahadevan L.C.** (1994) Mol. Cell. Biol., **14**(2), 1066-1074.
24. **Lee Y.S and Dutta A.** (2007) Genes Dev., **21**, 1025-1030.
25. **Madamanchi N.R., Zhou R.H., Vendrov A.E., Niu X.L., Runge M.S.** (2010) Circ. Res., **107**, 940-942.
26. **Mandal S., Lindgren A.G., Srivastava A.S., Clark A.T., Banerjee U.** (2011) Stem Cells, **29**, 486-495.
27. **Mao L., Wertzler K.J., Maloney S.C., Wang Z., Magnuson N.S., Reeves R.** (2009) Mol. Cell Biol., **20**, 5426-5440.
28. **Mercer J.R., Cheng K.K., Figg N., Gorenne I., Mahmoudi M., Griffin J., Vidal-Puig A., Logan A., Murphy M.P., Bennett M.** (2010) Circ. Res., **107**, 1021-1031.
29. **Misteli T., Gunjan A., Hock R., Bustin M., Brown D.T.** (2000) Nature, **408**, 877-881.
30. **Pedersen P.L.** (1978) Prog. Exp. Tumor Res., **22**, 190-274.
31. **Pedulla M.L., Treff N.R., Resar L.M.S., Reeves R.** (2001) Gene, **271**, 51-58.
32. **Penchovsky R., Chakarov S., Genova G.** (1998) Compt. Rend. Acad. Bulg. Sci., 51 (7-8), 83-86.
33. **Razzouk L. and Muntner P**. (2009) Curr. Hypertens. Rep., **11**, 127-132.
34. **Reeves R. and Nissen M.S.** (1990) J. Biol. Chem., **265**, 8573-8582.
35. **Reeves R., Langan T.A., Nissen M.S.** (1991) Proc. Natl. Acad. Sci. U. S. A ., **188**, 1671-1675.
36. **Reeves R. and Beckerbauer L** (2001) Biochim. Biophys. Acta, **1519**, 13-29.
37. **Roy L.M., Jaruga P., Wood T.G., McCullough A.K., Dizdaroglu M., Lloyd R.S.** (2007) J. Biol. Chem., **282**, 15790-15798.
38. **Sgarra R., Rustighi A., Tessari M.A., Di Bernardo J., Altamura S., Fusco A., Manfioletti G., Giancotti V.** (2004) FEBS Lett., **574**, 1-8.
39. **de Souza-Pinto N.C., Mason P.A., Hashiguchi K., Weissman L., Tian J., Guay D., Lebel M., Stevnsner T.V., Rasmussen L.J., Bohr V.A.** (2009) DNA Repair (Amst)., **8**, 704-719.
40. **Stuart J.A., Hashiguchi K., Wilson D.M. 3rd, Copeland W.C., Souza-Pinto N.C., Bohr V.A.** (2004) Nucleic Acids Res., **32**, 2181-2192.
41. **Tencheva Z., Velichkova A., Chakarov St.** (1995) Compt. Rend. Acad. Bulg. Sci., 48 (6), 101-104.
42. **Vartanian V., Lowell B., Minko I.G., Wood T.G., Ceci J.D., George S., Ballinger S.W., Corless C.L., McCullough A.K., Lloyd R.S.** (2006) Proc. Natl. Acad. Sci. U.S.A., **103**, 1864-1869.
43. **Zhao K., Käs E., Gonzalez E., Laemmli U.K.** (1993) EMBO J., **12**, 3237-3247.
44. **Zhelev N.Zh., Kardalinou E., Hazzalin C.A., Cano E., Barratt M.J., Mahadevan L.C.** (1993) Biochem. Soc. Trans., **21**, 907-911.
45. **Zhou X., Benson K.F., Ashar H.R., Chada K.** (1995) Nature, **376**, 771-774.