High-Mobility Group A (HMGA) Proteins and Breast Cancer

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Key Words

HMGA · Breast cancer · Prognostic factors · Blood samples

Summary

The high-mobility group A (HMGA) protein family includes HMGA1a, HMGA1b and HMGA1c, which are encoded by the same gene through alternative splicing, and the closely related HMGA2 protein. HMGA proteins have been found to be abundant in several malignant neoplasias, including colorectal, prostate, cervical, lung, thyroid and breast carcinoma. HMGA proteins can be ideal candidates for the identification of new prognosis and diagnosis factors with non-invasive methods. To provide some clarity regarding the abundance of articles on this topic, here we focus on the relationship between HMGA proteins and breast cancer and their clinical perspective in the development of new therapeutic strategies.

Schlüsselwörter

HMGA · Brustkrebs · Prognosefaktoren · Blutproben

Zusammenfassung

Die High-Mobility Group A (HMGA)-Proteinfamilie umfasst HMGA1a, HMGA1b und HMGA1c, die alle von einem einzigen Gen durch alternatives Spleißen kodiert werden, sowie das nah verwandte HMGA2-Protein. In einer Reihe von malignen Neoplasien, z.B. bei Kolorektal-, Prostata-, Zervix-, Lungen-, Schilddrüsen- und Mammarkarzinomen, konnte das HMGA-Protein in großen Mengen nachgewiesen werden. HMGA-Proteine sind ideale Kandidaten für die Identifikation von neuen Prognose- und Diagnosefaktoren mit nichtinvasiven Methoden. Um im Hinblick auf die große Anzahl von Artikeln zu diesem Thema ein wenig Klarheit zu schaffen, konzentrieren wir uns hier auf den Zusammenhang zwischen den HMGA-Proteinen und Brustkrebs und auf ihre klinische Bedeutung bei der Entwicklung von neuen therapeutischen Strategien.

HMGA Genes: Structure and Function

The high-mobility group (HMG) proteins are non-histone nuclear proteins known as ‘architectural transcription factors’. They have a high content of charged amino acids and a molecular mass of <30 kDa. The HMG protein family has been classified into 3 subfamilies: HMGA, HMGB, and HMGN, previously known as HMGI/Y, HMG1/2, and HMG14/17, respectively [1]. HMG proteins can bind to specific structures in DNA or chromatin in a sequence-independent manner, since

they recognize structure rather than a particular nucleotide sequence. Each of the subfamilies has a characteristic functional sequence motif: the ‘AT-hook’ for the HMGA family, the ‘HMG-box’ for the HMGB family, and the ‘nucleosomal binding domain’ for the HMGN family. Through this characteristic functional sequence motif, HMG proteins are able to modify the structure of their binding partners, generating a conformation that facilitates and enhances various DNA-dependent activities, such as transcription, replication, recombination, and repair [2]. The HMGA protein family includes

HMGA1a (107 amino acids, 11.7 kDa), HMGA1b (96 amino acids, 10.6 kDa) and the more recently identified HMGA1c (179 amino acids, 19.7 kDa), which are encoded by the same gene through alternative splicing, and the closely related HMGA2 protein (109 amino acids, 12 kDa). Chromosome mapping studies have located the HMGA1 gene on human chromosome 6 (6p21); the gene contains 8 exons which are distributed over a region of about 10 kb [3]. The human HMGA2 gene is located at chromosomal band 12q14–15 and contains at least 5 exons dispersed over a genomic region much larger than that of HMGA1 (≥ 160 kb), mainly because of longer 5' and 3' untranslated regions and because of the extremely long third intron of HMGA2 [4]. The HMGA1a and HMGA1b isoforms differ by only 11 amino acids present in HMGA1a but not in HMGA1b, and are encoded by the most abundant splice variants of the HMGA1 gene. HMGA1c is produced from the HMGA1 gene by alternative splicing using noncanonical splice donor and acceptor sites. This alternative splicing results in a frame shift so that the two proteins are identical in their first 65 amino acids but differ thereafter. In normal cells, transcripts from the HMGA2 gene code for the full-length HMGA2 protein.

As previously told, HMGA proteins have roles in assembling or modulating macromolecular complexes that are involved in various biological processes; they can bind to specific structures in DNA, modifying its conformation and consequently facilitating the binding of a group of transcription factors, as has already been shown for the interferon (IFN) gene [5, 6].

The HMGA proteins can also influence gene transcription by directly interacting with a transcription factor, modifying its conformation and enhancing its affinity to DNA [7].

Finally, the HMGA proteins have the ability to alter chromatin. In fact, HMGA may be associated with specific segments of genomic DNA that have a high affinity for the nuclear matrix and which are enriched in AT (matrix- and scaffold-associated) regions. The binding of HMGA proteins to these regions derepresses transcription by displacement of histone H1 by DNA [8].

HMGA Clinical Perspective

The role of HMGA proteins in embryogenesis, cell proliferation, differentiation, apoptosis and cancer development has been largely demonstrated [9]. HMGA proteins seem to play their major physiological role during embryogenesis; therefore, their expression is very high during embryonic development and very low or absent in adult tissue [10], but they have been found in abundance in several malignant neoplasias, like colorectal, prostate, cervical, lung, thyroid and breast carcinomas [9].

Breast cancer represents the main cause of morbidity and mortality in women throughout much of the developed world.

At least 10% of these tumors develop in families with strong aggregations of both breast and ovarian cancer, showing an autosomal-dominant transmission pattern [11]. BRCA1 and BRCA2 mutations are responsible for 15–20% of site-specific breast cancer families and the majority of breast and ovarian cancer families [12, 13]. Hereditary breast carcinomas occurring in BRCA1 patients have distinct histopathologic and immunophenotypic features. In fact, they generally show a higher grade, a pushing-margin growth pattern, negativity for estrogen receptor (ER), progesterone receptor (PR) and Erb-B2 (HER2) expression, and a high proportion of lymphocytic infiltration compared with sporadic and familial non-BRCA1/2 breast cancer, and are frequently associated with a poor prognosis [14].

Conversely, BRCA2 tumors are not clearly associated with a specific subtype, but invasive lobular, pleomorphic lobular, tubular and cribriform forms have been reported more frequently in this group [15, 16]. However, even though there is some evidence of an association between poor prognostic factors and BRCA1/2 mutations, it is still a matter of discussion whether the prognosis of familial breast cancer differs from that of sporadic cases.

Increased expression of the HMGA1 gene in human breast carcinoma has been demonstrated, with a direct correlation between HMGA1 protein levels and the metastatic phenotype of human breast cancer cell lines [17]. Chiappetta et al. [18] have shown that HMGA1 was not expressed in normal breast tissue whereas HMGA1 staining was intense in 25% of the hyperplastic lesions with cellular atypia and in 60% of the sporadic ductal carcinomas. They also investigated whether HMGA1 expression is correlated with Erb-B2, finding that there is a correlation between the overexpression of HMGA1 and Erb-B2. It could be hypothesized that HMGA1 proteins would be able to induce c-erbB2 expression by acting on its promoter or, alternatively, that the activation of the Erb-B2 transduction pathway may lead to increased HMGA1 protein synthesis [18].

Several reports have demonstrated that BRCA1 protein levels are decreased in a subset of sporadic breast carcinomas compared to normal breast tissues [19]. One possible mechanism for the low levels of BRCA1 expression in breast carcinomas could be hypermethylation of the BRCA1 promoter. The methylation status of the BRCA1 promoter in sporadic breast cancer specimens was extensively investigated [20] and it seems to explain only a small number of breast cancer cases. Similarly, loss of heterozygosity at the BRCA1 locus proved insufficient as an explanation for the low levels of BRCA1 expression in breast carcinomas, since studies showed that loss of heterozygosity correlated with reduced BRCA1 expression in only a few cases [21]. Baldassarre et al. [22] have demonstrated that HMGA1b protein inhibits BRCA1 promoter activity in both human and mouse genes by directly binding to the promoter regions, suggesting a possible and more reasonable mechanism responsible for the low BRCA1 protein levels.

Another way by which HMGA may have a role in cancer progression is shown in breast carcinomas: The overexpression of HMGA1 can inactivate the p53 apoptotic activity by promoting the cytoplasmatic relocation of the p53 proapoptotic activator HIPK2 [23]. Chiappetta et al. [24] have also analyzed whether HMGA1 detection might have a prognostic role in familial breast carcinomas, showing that HMGA1 overexpression is less frequent in BRCA1 patients in comparison to sporadic, BRCA2 and BRCA3 (negative for mutations in both genes) breast carcinoma patients. Moreover, HMGA protein expression does not seem to correlate with a bad prognosis in familial breast carcinoma patients. By contrast, it might even represent a good prognostic factor for breast cancer patients carrying a mutated BRCA2 gene.

In various studies, it has been proposed that a link exists between HMGA proteins and tumor cell response during chemotherapy. It has been seen that expression of HMGA2 intensifies the cytotoxic effect induced by the chemotherapeutic agent cisplatin [25], and Summer et al. [26] have found that HMGA2 protects cancer cells from certain DNA-damaging agents used in cancer treatment like hydroxyurea (Hu), but not from other kinds of chemotherapeutic drugs like paclitaxel. So the protective effects exerted by HMGA2 seem to be limited to particular repair pathways. Another piece of evidence for the relationship between HMGA proteins and tumor cell response during chemotherapy is given by analyzing BRCA expression and cis-platinum (cisplatin) sensitivity: Exposure to cisplatin leads to the up-regulation of BRCA1 mRNA expression in mammary carcinoma cells [27], and BRCA1 protein levels positively correlate with cisplatin resistance in breast and ovary carcinoma cells [28]. Recently, Brca1 null mouse embryonic stem (ES) cells were demonstrated to be more sensitive to cisplatin as compared to their normal counterparts [29]. Therefore, Baldassarre et al. [30] have investigated the role of HMGA1 protein following cisplatin-induced DNA damage, both in the breast carcinoma cell line MCF-7 overexpressing the HMGA1b protein and in ES cells null for the Hmga1 gene. They have demonstrated that high expression levels of HMGA1 proteins in MCF-7 or mouse ES cells resulted in enhanced sensitivity to cisplatin and bleomycin, likely by leading to reduced BRCA1 protein levels, thus suggesting that inhibition of BRCA1 function by high expression levels of HMGA1 protein could represent a mechanism for cancer cells to reach a more aggressive phenotype [30]. All these data support the hypothesis that HMGA proteins can be used for disease diagnosis, the choice of treatment regimens and the future development of anticancer drugs.

HMGA Detection in Peripheral Blood Samples

In order to improve the survival rate in breast cancer, numerous research teams are trying to develop techniques to detect metastasis or recurrent disease in preclinical or presympto-

matic phases. In recent years, various reports have identified free circulating nucleic acids in the peripheral blood of patients affected by lung cancer, breast cancer and colon cancer. Qualitative studies have shown alterations in nucleic acids extracted from the plasma of patients that are similar to alterations found in DNA and RNA of primary tumors, suggesting that plasma and serum nucleic acids originate from tumor cells [31, 32].

DNA and RNA were found in the plasma and sera from healthy donors and cancer patients, and increased levels were observed in affected groups. Further, in breast cancer, a statistical relationship between poor prognosis and the presence of tumor nucleic acids has been reported [33]. Prognostic factors and several molecular markers were evaluated in association with established histologic and clinical prognostic parameters of breast cancer; considerable interest in HMGA proteins has been stimulated by the observation that they are involved in the fundamental biological processes of cell proliferation and differentiation [9].

Little is known about the origin and protective mechanism of free nucleic acids in plasma and the release mechanisms of these molecules from tumor cells into the bloodstream. In order to analyze the nature and origin of nucleic acids in plasma and culture media samples, various RNA extraction protocols have been performed; at the same time, these processes were accompanied by imaging techniques, such as confocal microscopy and flow cytometry to consolidate the experimental protocols. The results suggested that most of the RNA detected in plasma as free nucleic acids could be protected within complexes formed by nucleic acids and some membrane glycopeptides. In fact, the data obtained after ultracentrifugation showed that RNA as free nucleic acid is soon degraded in plasma and cannot be isolated by ultracentrifugation, supporting the idea that RNA extracted from plasma must be protected in the multiparticle complex [34].

The expression of HMGA2 in free plasma RNA has already been examined in peripheral blood samples from breast cancer patients and compared to healthy donors, using a haem-nested reverse transcriptase-polymerase chain reaction (RT-PCR) technique [35]. In these studies, HMGA2 expression was not detectable in any of the samples from healthy volunteers, whereas expression was shown in the peripheral blood of patients affected by breast cancer. Sezer et al. [35] reported the expression of HMGA2 in the peripheral blood of breast cancer patients and, interestingly, they have shown for the first time that this was restricted to patients with metastatic disease.

In later studies, HMGA2 mRNA expression was estimated in peripheral blood samples from patients with metastatic breast cancer, in order to establish a potential connection between HMGA2 levels and time of survival. Expression of HMGA2 was detected in the peripheral blood of 21 out of 69 patients with metastatic tumors. There was no statistically significant difference between the group of patients positive for HMGA2 expression and the group of patients without detect-

able HMGA2 in terms of age, time from diagnosis to first metastatic event, menopausal status, histologic type, hormone receptor status, and sites of metastases. The two groups were different regarding the receipt of chemotherapy, adjuvant or palliative chemotherapy, endocrine therapy or radiation therapy.

The results showed that median survival was 15.9 months in patients expressing HMGA2, 38% were still alive at the last follow-up contact, while in the group of patients without HMGA2 expression the median survival had not yet been reached and 85.4% were still alive at the last follow-up contact [36].

These studies demonstrate that HMGA2 is an important element in tumorigenesis; moreover, they underline the prognostic relevance of HMGA2 mRNA expression in the peripheral blood of breast cancer patients with metastatic disease

with respect to clinical pathologic parameters. In fact, HMGA2 expression is highly significant in breast cancer, and circulating HMGA2 mRNA is a powerful independent prognostic indicator for overall survival.

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Conflict of Interest

All authors declare to have no potential conflict of interest.

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