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HMGA1-pseudogene overexpression contributes to cancer progression

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Two pseudogenes for *HMGA1*, whose overexpression has a critical role in cancer progression, have been identified. They act as decoy for miRNAs that are able to target the *HMGA1* gene then enhancing cell proliferation and migration. Moreover, these pseudogenes contain sequences that are potential target sites for cancer-related miRNAs. Interestingly, *HMGA1* pseudogenes are highly expressed in human anaplastic thyroid carcinomas, that is one of the most aggressive tumor in mankind, but almost undetectable in well differentiated thyroid carcinomas.

The human genome has been sequenced, and no more than 2% codes for proteins building our bodies.[1] Therefore, a key question is to identify which is the meaning of the other 98% of the genome. Many evidences show that the mammalian genome is able to generate numerous previously undiscovered transcripts called "non coding RNA" (ncRNA).[1] However, the role of these ncRNAs remains largely unknown. The ncRNAs include different classes: the "short interfering RNAs" (siRNAs), the "Natural Antisense Transcripts" (NATs), the "microRNAs" (miRNAs), long non coding RNAs (lncRNAs), and pseudogenes.[2]

siRNAs are double-stranded small interfering RNAs of ~21 base pairs in length that serve as effector molecules of sequence-specific gene silencing. They are highly conserved across species.[3]

NATs are RNAs that are at least in part complementary to other endogenous RNAs. They might be transcribed *in cis* from opposing DNA strands at the same genomic locus (*cis*-NATs) or *in trans* at separate loci (*trans*-NATs).[4] NATs biological role is based on gene knockdown induced by base-pairing of sense and anti-sense strands. To date, in human have been identified cis-encoded exon-overlapping sense-antisense (SA) in a number of 7356 NATs.[4]

Among *trans*-NATs the most studied are miRNAs. They are small RNAs (18-21 nt) that can inhibit mRNA expression by binding to 3′ Untranslated Region (3′UTR) with perfect or imperfect match, suppressing mRNA translation or affecting RNA stability.[5] Krek et al. reported that vertebrate *microRNAs* target, usually, almost 200 transcripts each one.[6,7]

Long non coding RNAs (lncRNAs) are non-protein coding transcripts longer than 200 nucleotides.[8] This rather arbitrary limit distinguishes lncRNAs from small regulatory RNAs such as miRNAs, siRNAs, Piwi-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), and other short RNAs.[8]

Pseudogenes are dysfunctional relatives of genes that have lost their protein-coding skill or are otherwise no longer expressed in the cell.[9-11] They are characterized by a mixture of homology to a known gene and nonfunctionality: every pseudogene has a DNA sequence that is similar to some functional genes, but they are unable to produce functional final protein products.[9-11]

There are 3 major families of pseudogenes: processed (or retrotransposed), non-processed (or duplicated), and disabled pseudogenes. Processed pseudogenes originate from a segment of mRNA transcript of a gene that is spontaneously reverse transcribed back into DNA and inserted into chromosomal DNA. Once these pseudogenes are inserted back into the genome, they generally include a poly-A tail, and their introns are

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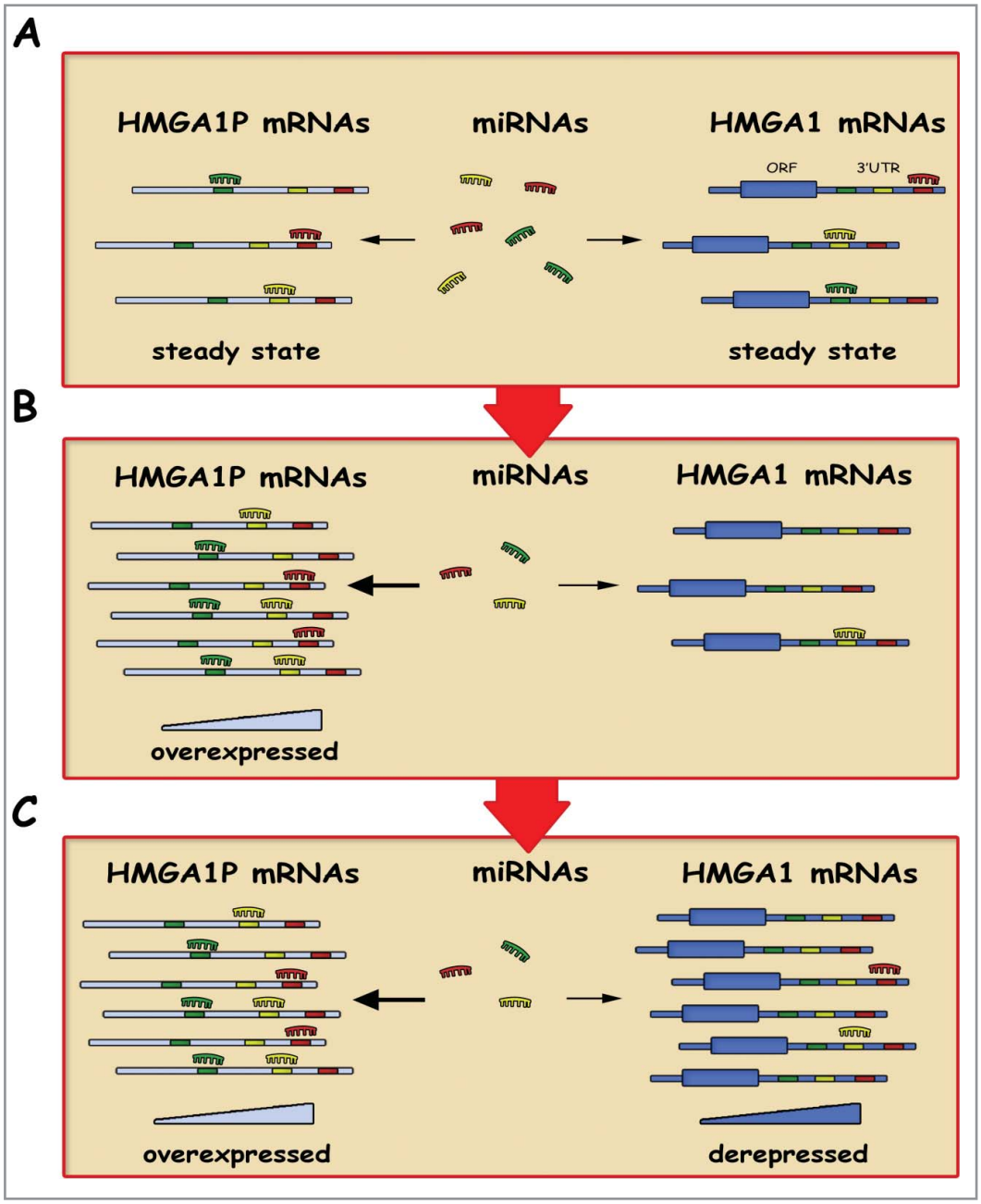

Figure 1. Decoy function of *HMGA1Ps*. Several RNA transcripts have seed sequences for the same miRNAs (**A**). As proposed by Esposito et al., overexpression of *HMGA1Ps* mRNAs increases cellular concentrations of particular seed sequences (**B**), resulting in the derepression of *HMGA1* transcripts that contain the same seed sequences (**C**).

frequently spliced out.[12] Non-processed pseudogenes arise as a consequence of a gene duplication event and then acquire mutations making them nonfunctional. Duplicated pseudogenes usually include all the same characteristics of genes from they originate, as well as an intact exon-intron structure and promoter sequences.[12] Disabled genes (or unitary pseudogenes) present different mutations that stop a gene from being productively transcribed or translated.[13]

Recently, Esposito et al. have identified 2 processed pseudogenes, *HMGA1P6* and *HMGA1P7*, belonging to the *HMGA1* gene family. They are placed at 13q12.12 and 6q23.2, respectively, and have been reported to have a critical role in the process of carcinogenesis.[14] The *High Mobility Group A1* (*HMGA1*) gene codes for the HMGA1 proteins, HMGA1a and HMGA1b.[15] These proteins are non-histone chromatinic proteins that bind to DNA and organize chromatin architecture, interacting with several transcription factors, and then regulate the expression of several genes, positively or negatively.[15] Their role in carcinogenesis is widely accepted. Indeed, these proteins are expressed at high levels for the duration of embryogenesis and in malignancies where their expression levels point out a poor prognosis of the cancer patients, whereas their expression is brought down in adult normal tissues.[16-18] Moreover, the *knock-down* of *HMGA1* expression prevents thyroid cells transformation and leads cancer cells of diverse tissue origin to apoptosis.[19-21] On the contrary, their overexpression *in vitro* induces mouse and rat fibroblast transformation,[22] and transgenic mice overexpressing *hmga1* develop several neoplasias including pituitary adenomas, Natural Killer (NK)/T-cell lymphomas,[24] lipomas, cervix and body adenocarcinomas.[15]

HMGA1 pseudogenes, *HMGA1P6* and *HMGA 1P7*, show just few mismatches all over the coding sequence and the 5' and 3' UTRs of *HMGA1*. They have conserved seed matches for miRNAs that have been previously confirmed to target the *HMGA1* gene. Subsequently, the authors show that these pseudogenes equally operate as decoys for *HMGA1*-targeting miRNAs (**Fig. 1**). Indeed, *HMGA1P6* and *HMGA1P7* overexpression increases HMGA1 protein levels whereas their silencing results in decreased *HMGA1* mRNA and protein levels.[14] Consistently

with the *HMGA1P6* and *HMGA1P7* decoy function, cells overexpressing them show an enhanced migration, invasiveness, and a faster proliferation ability.[14] Opposite results are obtained when these pseudogenes are silenced with also an increase in apoptotic cells following a reduced *HMGA1* protein levels, as already observed when *HMGA1* is knocked down in thyroid cells.[21]

The generation of transgenic mice overexpressing *HMGA1P6* or *HMGA1P7* has confirmed their oncogenic activity. Indeed, mouse embryonic fibroblasts (MEFs) deriving from *HMGA1P6* or *HMGA1P7*[14] overexpressing mice grow faster and senesce later than their wild-type counterparts.

However, we retain that the key point of this study is the finding of a role of these pseudogenes in human carcinogenesis. In fact, anaplastic thyroid carcinoma (ATC), that represents one of the most aggressive tumors in the mankind, evidenced a exceptionally high expression of the *HMGA1* pseudogenes essentially correlating with the HMGA1 protein levels.[14] Conversely, their expression is almost undetectable in papillary and follicular thyroid carcinomas, that are well differentiated and much less aggressive. Analogous results were obtained when *HMGA1Ps* expression was analyzed in human ovarian[14] and larynx carcinomas, and pituitary adenomas (manuscript in preparation).

Remarkably, the presence in the *HMGA1P6*, *HMGA1P7*, and also *HMGA1* UTR regions of sequences that are potential target sites for cancer-related miRNAs targeting genes such as *High Mobility Group A2 (HMGA2), Enhancer of Zeste Homolog 2 (EZH2), Vascular Endothelial Growth Factor (VEGF)*, and *Ephrin Type-A Receptor 3 (Epha3)*, that are effectively upregulated in *HMGA1P6* and *HMGA1P7*[14] overexpressing cells and MEFs with respect to the control cells. This has important consequences. In fact, it means that high *HMGA1* gene or its pseudogene expression allows an increase in HMGA2 and EZH2 protein levels then contributing to cancer progression.[14] Such a mechanism is likely to occur in ATC where the overexpression of *EZH2* has been detected in ATC but not in the undifferentiated thyroid carcinomas.[25,26]

Interestingly, Kumar et al. have recently demonstrated that the *HMGA2* 3′ UTR contains 7 conserved seed sequences for let-7, which has been previously demonstrated to constrain lung cancer development. They identified 6 ceRNA targets that are regulated by *hmga2* in a let7-dependent manner: Transforming growth factor β receptor III (tgfbr3), Angiopoietin-related protein 2 (Angptl2), *Fibronectin Type III Domain Containing Protein 3 (Fndc3)*, *Ski-like protein (Skil)*

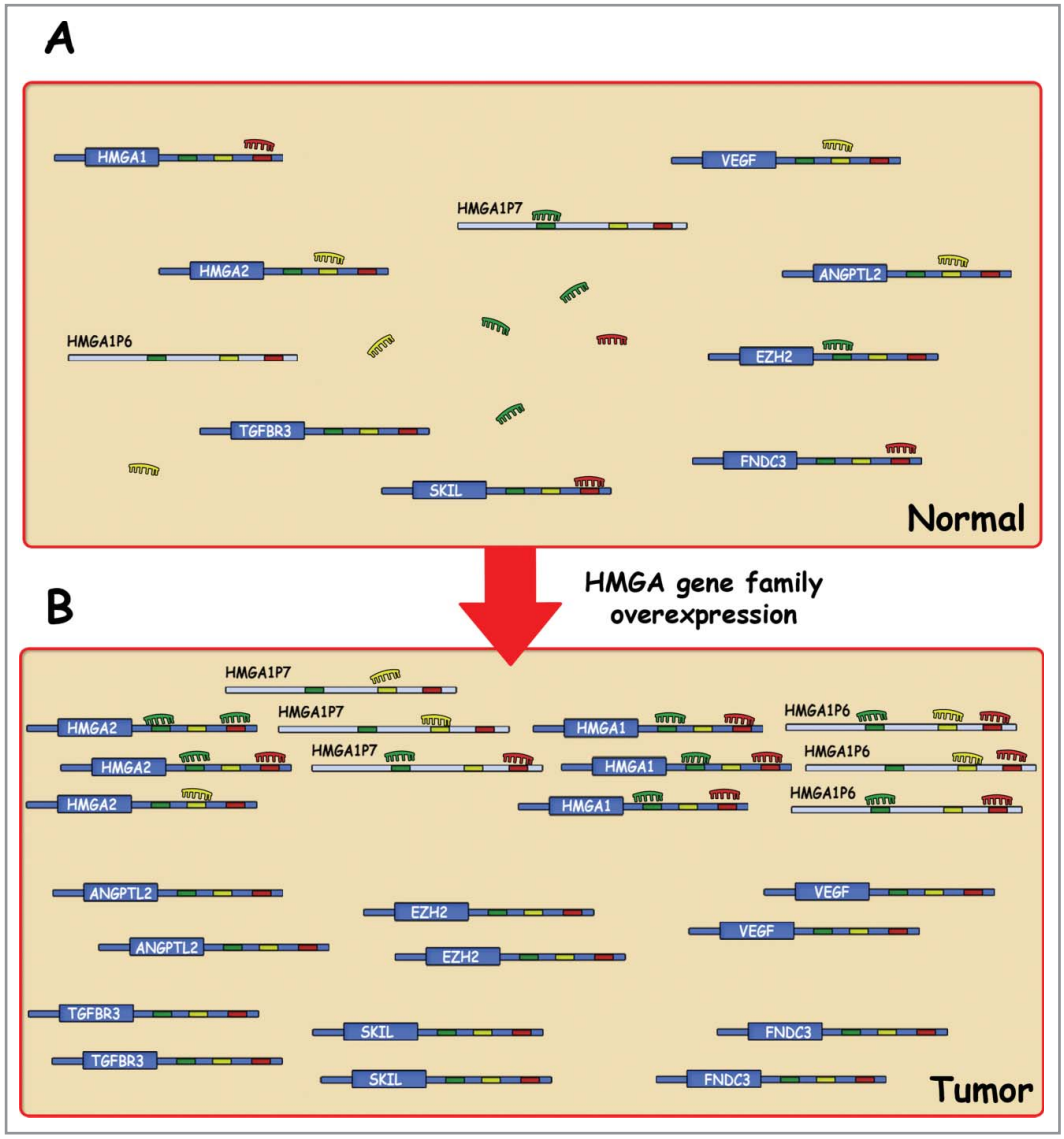

Figure 2. A ceRNA model for the *HMGA* gene family. *HMGA* RNA transcripts have seed sequences for the same miRNAs shared with other transcripts (**A**). As proposed by Esposito and Kumar, in malignancies, the HMGA gene family overexpression increases cellular concentrations of particular seed sequences, resulting in the derepression of several cancer-related gene transcripts that contain the same seed sequences (**B**).

and *Hmga1*.[27] Then, on the basis of these results also *HMGA1*, through its 3'UTR, may function as decoy for *HMGA2* and other cancer-related gene expression. Therefore, a synergism between members of HMGA protein family might be envisaged based not only on common functions but also on the ability of the *HMGA* mRNAs to act as decoy for miRNAs able to target themselves.

In conclusion, the results published by Esposito et al. indicate that also the expression of the *HMGA1* pseudogenes contribute to carcinogenesis and, together with the paper by Kumar et al., reveal another mechanism by which *HMGA* gene family has a critical role in cancer progression based on the ability to regulate gene expression also as non-coding RNAs (**Fig. 2**). Therefore, these new reports make even more important the *HMGA* gene family in cancer diagnosis and prognosis, and as potential target for an innovative cancer therapy.

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