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Research Article

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Cancer Stem Cells Regulation with a Sublingual Nanotherapy using Ultra Low Doses of Non-Coding RNAs

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Abstract

Tumors are heterogeneous tissues with abundant phenotypically and functionally distinct cell subpopulations, each having different capacities to grow, differentiate, develop drug resistance and form metastases. Tumors contain a functional subpopulation of cells that exhibit stem cell properties. These cells, named cancer stem cells (CSCs), play significant roles in the initiation and progression of cancer. So far, CSCs have been identified in breast, pancreatic, prostate, colon, head and neck, ovarian and liver cancers, melanoma and brain tumors. CSCs are defined by the following properties: (a) unlimited self-renewal capacities, (b) the ability to differentiate into non-CSC daughter cells, (c) high tumorigenicity upon injection in immunocompromised mice, and (d) have remarkable resistance to conventional therapies. MicroRNAs or miRNAs are short non-coding RNAs that regulate gene expression at the post-transcriptional level by leading to the degradation of target mRNA or repression of mRNA translation. Recent studies have highlighted several miRNAs to be differentially expressed in normal and cancer stem cells and established their role in targeting genes and pathways supporting cancer stemness properties. Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs that have no potential to code proteins and are more than 200 nucleotides in length. LncRNAs can act at the transcriptional, posttranscriptional and translational level. As such, they may be involved in various biological processes such as DNA damage repair, inflammation, metabolism, cell survival, cell signaling, cell growth and differentiation. Accumulating evidence indicates that lncRNAs are key regulators of the CSCs subpopulation, thereby contributing to cancer progression. These non-coding RNA molecules represent, of course, particularly attractive targets for regulating CSCs; for this purpose, we have developed a sublingual nanotherapy delivered without any undesirable side effects thanks to the use of ultra-low doses.

Keywords: Cancer Stem Cells, Long Non-Coding RNAs, MicroRNAs, Sublingual Nanotherapy

Abbreviations

CSCs- Cancer Stem Cells
BI(G)MED: Bio Immune(G)ene Medicine
ALDH1: aldehyde dehydrogenase1
ncRNAs: non-coding RNAs
lncRNAs: long non-coding RNAs
lincRNAs: long intergenic ncRNAs

Introduction

Cancer stem cells (CSCs) also known as tumor initiating cells (TICs) are a small subpopulation (0.05-1%) of tumor cells that have been identified in most types of cancer [1]. The CSCs theory of cancer progression presents tumor as a hierarchically organized tissue with CSCs population at the top rank in the hierarchy, that then generate the more differentiated bulk of the tumor cells with lower or limited proliferative potentials [2]. Stem cells are characterized by their capacity for self-renewal and their ability to differentiate into diverse specialized cell types. This concept has been extended from the embryonic stem cells (ESCs) and adult stem cells to cancer stem cells (CSCs) and induced pluripotent stem (IPS) cells [3] (Figure 1).

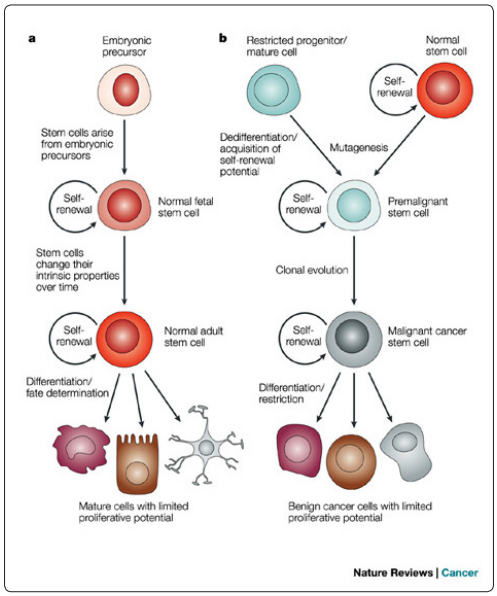

Figure 1: Parallels between normal stem cells and cancer stem cells

Thus, in general, we can say that features distinguishing cancer

stem cells from the bulk of other tumor cells include pluripotency, self-renewal capacity, low proliferation rate, and tumor-initiating ability (Figure 2).

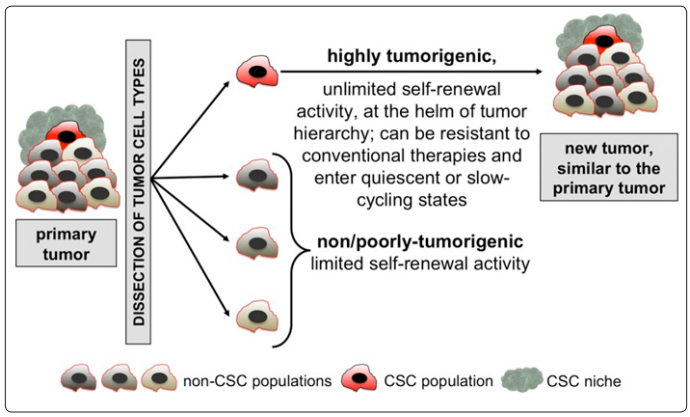

Figure 2: The classical "cancer stem cell" (CSC) concept

CSCs are highly malignant, as they confer drug resistance and facilitate tumor progression, relapse, and metastasis [3,4]. Because of their resistance to conventional cancer treatments, CSCs are of considerable interest in clinical practice [5,6]. These cells are endowed with:

- the capacity of **long-term proliferation**,
- ability to undergo **symmetric cell division**
- and seed new tumors by virtue of their **intrinsic self-protection** and self-renewal capabilities.

Normal stem cells and **CSCs** share different identical biological properties, and according to this similarity, CSCs are commonly characterised by the expression of surface markers associated with stem cells, such as CD133, CD44, CD90 [2]. But accumulation of genetic and epigenetic alterations deregulates the basic stem cell biology and distinguish **CSCs** from the normal stem cells in their chemoresistance, enhanced tumorigenic and metastatic activities [4].

In adults, stem cells reside in a physiologically limited and specialized microenvironment, or **niche**, that supports stem cells but varies in nature and location depending on the tissue type. The **stem cell niche** is defined as a microenvironment that anchors SCs to maintain their stemness and in adult somatic tissues plays an **essential role in maintaining stem cells or preventing tumorigenesis** by providing primarily inhibitory signals for both proliferation and differentiation.

The **cancer-inducing niche** can be explained as the continuous irregular environment that is typically recognized as **chronic inflammation** even if all the components and mechanisms of this inflammation are not yet explained (Figure 3) [7].

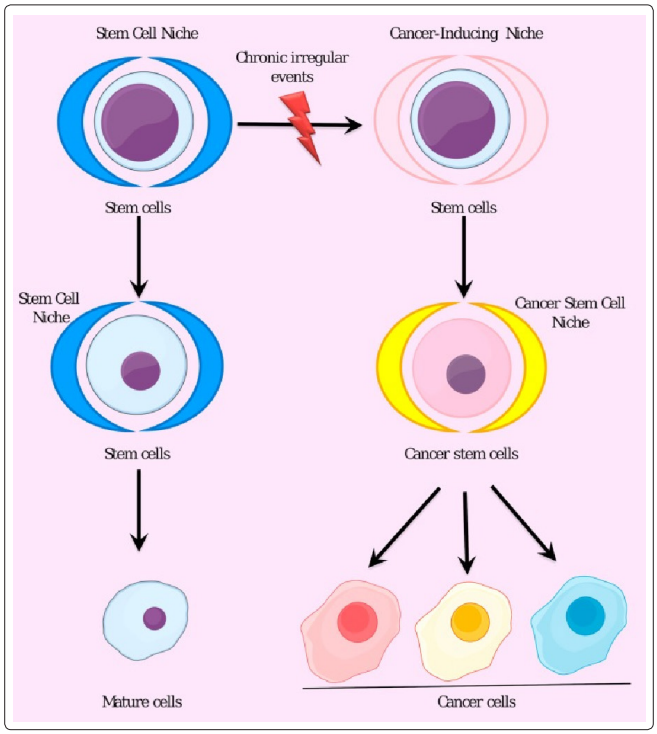

Figure 3: Stem cell differentiation in the stem cell niche and cancer-inducing niche.

Loss of the niche can lead to loss of stem cells, indicating the reliance of stem cells on niche signals. Therefore, **cancer stem cells (CSCs)** may arise from an intrinsic mutation, leading to self-sufficient cell proliferation, and/or may also involve deregulation or alteration of the niche by dominant proliferation-promoting signals. Regarding the **origin of the CSCs**, there are currently three hypotheses, none of them eliminating the others (Figure 4) [8]:

- first hypothesis: CSCs arise from stem cells
- second hypothesis: they arise from progenitor cells
- third hypothesis: they arise from differentiated cells

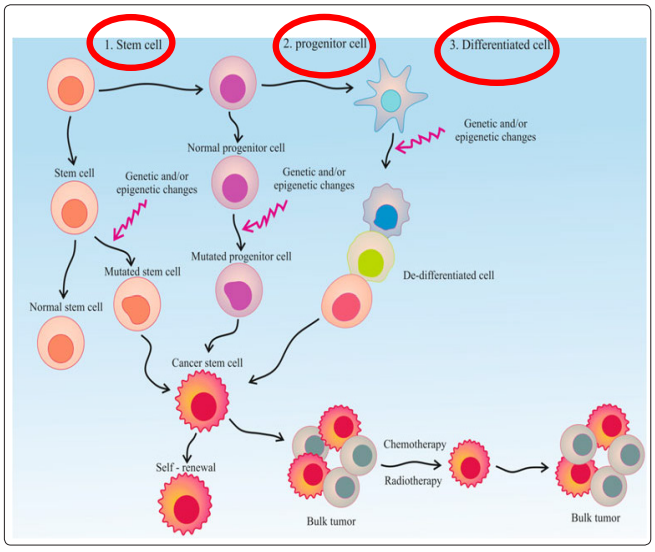

Figure 4: Three hypotheses about the origin of cancer stem cells

Large numbers of **CSC**s have been isolated using cell surface markers in addition to those already mentioned above such as CD24, CD29, aldehyde dehydrogenase1 (ALDH1) and epithelial-specific antigen (ESA). Remarkably, however, the expression of **CSCs** surface markers is cancer type-specific or cancer subtype-specific as it has been showed by *Hao & al* in 2014 [9]. Stem cell biology is primarily controlled by ***four major signal transduction*** pathways: the **Wnt/β-catenin, Notch, Hedgehog, and BMI-1** pathways (Figure 5). It has been proven that alterations in these molecular pathways can provide an unrestricted proliferative capability to **CSC**s [10,11].

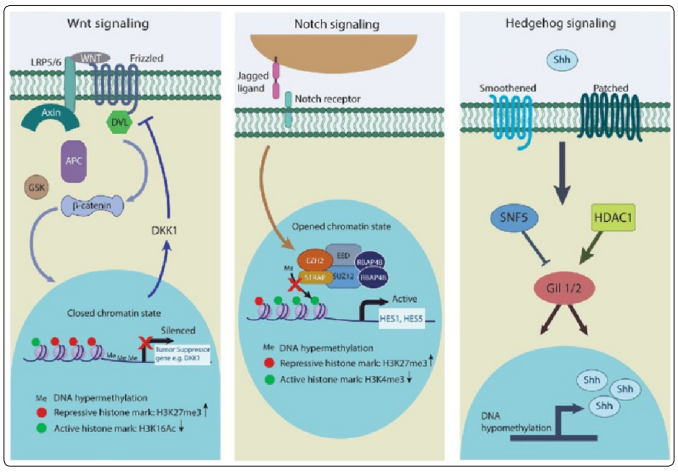

Figure 5: Regulation of key cancer stem cell signaling pathways by epigenetic mechanisms

Targeting **CSCs** is vital for treating cancer effectively, in particular to prevent tumor relapse. Currently, multiple strategies are being considered for the specific purpose of eradicating **CSCs** and their niche [12]. These include targeting-specific surface markers, modulating signaling pathways, interfering with microenvironment signals, inducing CSCs apoptosis and differentiation, inhibiting drug efflux pumps and altering miRNA expression.

Materials and methods

In the context of Bio Immune(G)ene Medicine, abbr. **BI(G)MED**, we choose the option of epigenomic regulation using **non-coding RNAs (ncRNAs)**, and especially **microRNAs** and **long non-coding RNAs** but also **circular RNAs**.

Several lines of evidence have shown the critical roles of **ncRNAs** in **CSCs** biology [13-22]. Accumulating evidence has provided insights into the importance of **ncRNA**s as regulators in several critical steps of cancer development, such as carcinogenesis, cancer invasion, and metastasis.

microRNAs and cancer stem cells

On a general level, miRNAs are associated with tumor initiation and development through regulating CSC characteristics including the ability of self-renewal, drug resistance, and tumorigenicity as shown on (Figure 6) [13].

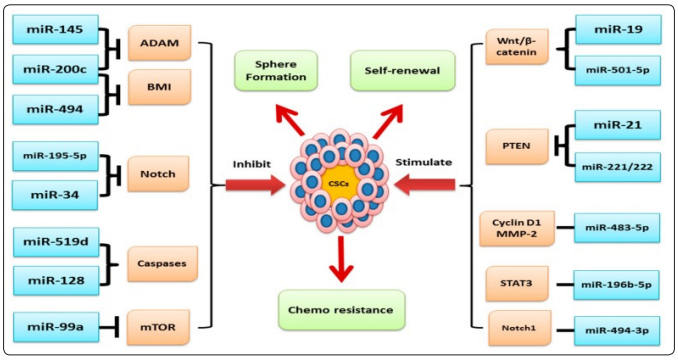

Figure 6: miRNAs in regulating cancer stem cells characteristics

Key microRNAs have been described to affect the main signaling pathways regulating CSCs.
It was for example showed that different microRNAs affect [14]:
- *Wnt signaling* → for instance miR-600 or miR-217
- *Notch signaling* → miR-34a or miR-141
- *Hedgehog signaling* → miR-324-5p or miR-326

miRNAs are key intracellular regulators of gene expression and play roles in tumor initiation and distant metastasis via controlling CSCs properties. Some of these miRNAs have *tumor suppressor* properties, whereas others act as *oncogenes* [13]. For example, miR-15a, miR-16-1, miR-34, miR-200c, and let-7 ***act as tumor suppressor*** in various types of ***CSCs and are commonly downregulated*** in different cancers [15]. Upregulation of these miRNAs reduces expression of oncogenic and antiapoptotic proteins. In contrast, miR-21, miR-155, and the miR-17 miR-92 cluster ***are oncogenic and are upregulated*** in various types of cancer [16].

All these data show how important it could be to use **microRNAs** on a therapeutic level with the aim to inhibit so called **oncomiRs** and restore **tumor suppressor microRNAs** (Figure 7) [17].

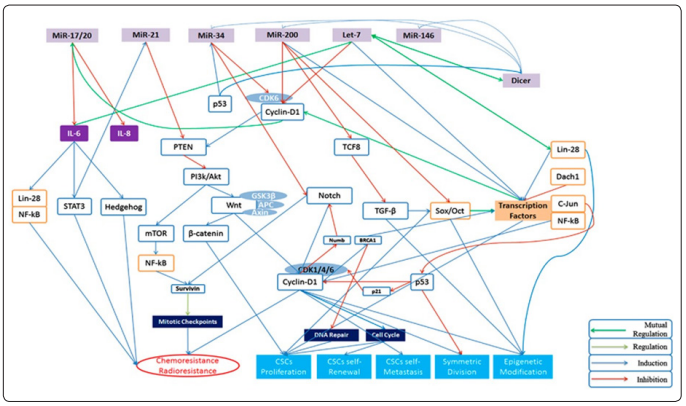

Figure 7: The networks of miRNAs regulation in cancer cells and CSCs

Long non-coding RNAS and cancer stem cells

Increasing evidence has suggested that the **miRNA** function is influenced by long noncoding RNAs **(lncRNAs)**. **LncRNAs** are a class of transcripts that are longer than 200 nucleotides and have a restricted capability for protein coding [18]. About 80% of the transcription in mammalian genomes is exclusively associated with **lncRNAs**. The ENCODE consortium (version 18) has confirmed the existence of 13,562 lncRNAs, and approximately 2/3 of them

are located between genes, which are termed long intergenic ncRNAs **(lincRNAs)**. Others include overlapping, antisense, and intronic lncRNAs. At present, accumulated data reveal that lncRNAs are significantly involved in the control of multiple cellular processes [23].

Similar to protein-coding genes, **lncRNAs** vary considerably in function. ***The function of lncRNAs often relates to the transcriptional regulation of genes*** leading to differential mRNA processing. There are different ways by which ***lncRNAs*** function to regulate target gene expression according to their nucleic or cytoplasmic location [24].

In the nucleus, **lncRNAs** regulate gene expression by controlling the local chromatin structure or recruiting regulatory molecules to specific loci. In the cytoplasm, lncRNAs interact with other types of RNA, and affect functions including mRNA stability, mRNA translation, or microRNA sponge (Figure 8).

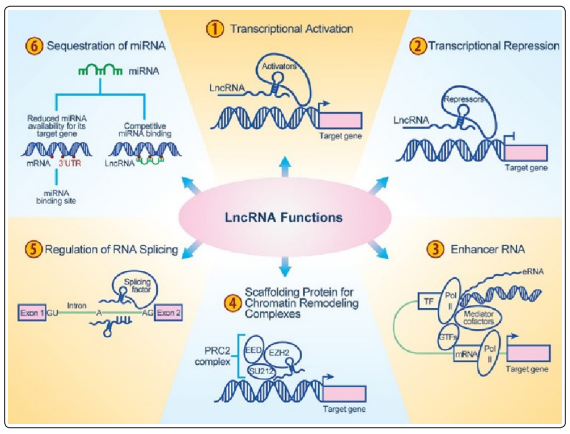

Figure 8: LncRNA Functions

LncRNAs work mainly in four modes: **signal**, **decoy**, **guide** and **scaffold** (Figure 9):

- **Signal:** LncRNAs can signal the space, time, and expression of gene transcription to modulate transcription factors and signaling pathways. LncRNAs directly bind to nucleic acid to inhibit the downstream molecule transcription named signal work model.
- **Decoy:** LncRNAs can bind and titrate away the protein or RNA target. LncRNAs combine with protein and then bind to nucleic acid to inhibit the downstream molecule transcription named decoy work model.
- **Guide:** LncRNAs can guide RNA-binding proteins to special target genes. Proteins guide LncRNA to bind to nucleic acid to inhibit the downstream molecule transcription named guide work model.
- **Scaffold:** LncRNAs can assemble different proteins to form complexes to initiate the special biological functions. Different proteins and LncRNAs combine together to bind to nucleic acid to inhibit the downstream molecule transcription named scaffold work model

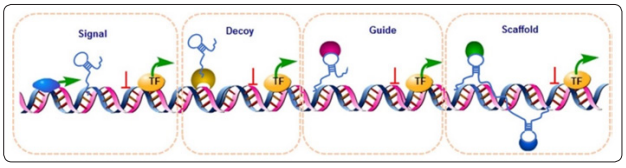

Figure 9: Four models of LncRNAs

lncRNAs have also emerged as important regulators in oncogenic and tumor suppressor pathways. Accumulating evidence provides mechanistic insight demonstrating how **lncRNAs** ***regulate important cellular signaling pathways in cancer cells*** at transcriptional, post-transcriptional, and epigenetic levels [25]. Moreover, numerous lncRNAs could be associated to the primary well-known so-called, ***cancer hallmarks***" (Figure 10).

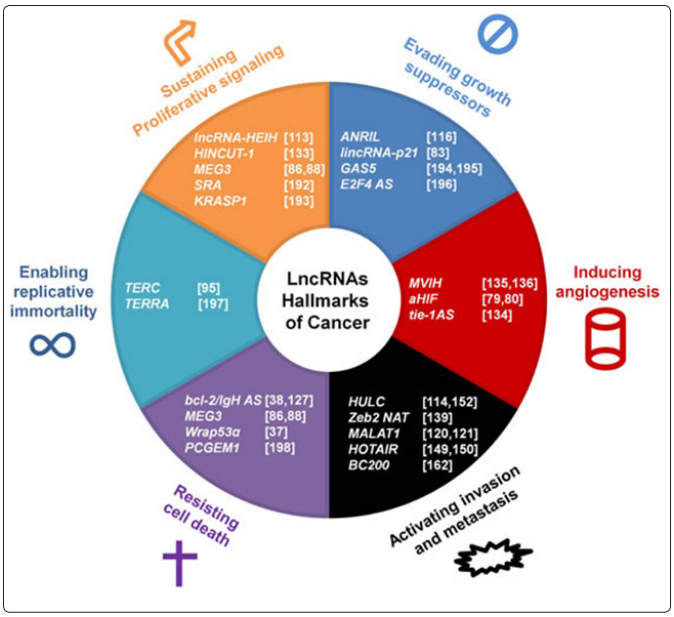

Figure 10: LncRNAs impact the hallmarks of cancer. The six hallmarks of cancer are shown with selected associated lncRNAs that are involved in cancer onset and progression

The small set of key transcription factors, including SOX2, Oct4 and Nanog, known as pluripotency transcription factors, are also being recognized to be highly important to the generation of **CSCs**, and also to function in the activation of **EMT** during tumorigenesis [26]. Various EMT-regulating **lncRNAs** have now been found to ***promote the CSC phenotype*** through regulation of these pluripotent stem cell transcription factors (Figure 11).

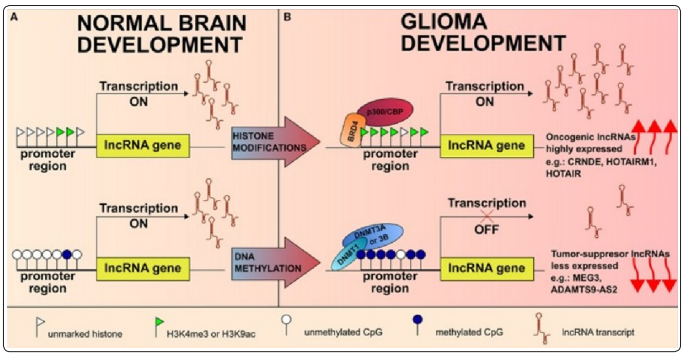

Figure 11: Examples of Epigenetic Modulation of Dysregulated LncRNAs Expression in Glioma

These data and an increasing number of studies have shown that **lncRNAs are either up- or down-regulated in cancers**, and also that it's possible to use **long noncoding RNAs** on a therapeutic level with the aim to inhibit especially **CSCs** and resulting **EMT** [27].

Results

The therapeutic application of **ncRNAs** cannot be conceived otherwise than by using **ultra-low doses** in the context of a nanotherapy as currently represented by the BI(G)MED. This is made possible by the principle of **Hormesis**, borrowed from toxicology, and applied to nanodoses, which explains the **rule of reversal of action according to the dose** (Figure 12) [28,29].

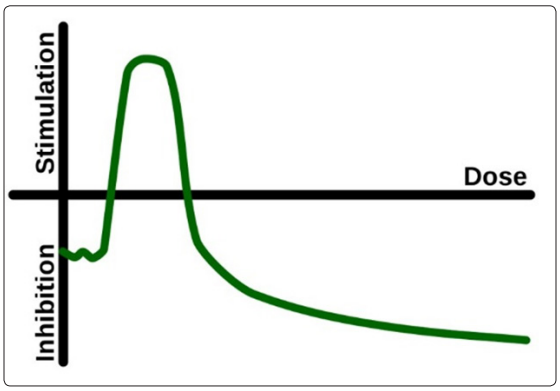

Figure 12: Principle of Hormesis

The application of these different principles and rules allows us to simultaneously use a large number of non-coding RNAs according to their validated functions and roles in the treatment of cancerous tumors via stem cells.

Table I: Example of the Bio Immune Regulator for CSCs

CODE	COMPOSITION	DILUTION
STEMREG	Sox2 TF	$1X10^{-14}$ mol
	Nanog gene	"
	Beta Catenin	"
	miR-17-92	"
	miR-21	"
	miR-34	$1X10^{-5}$ mol
	miR-125b	"
	miR-290	$1X10^{-9}$ mol
	Lnc RNA HOTAIR	$1X10^{-14}$ mol
	Lnc RNA GAS5	$1X10^{-5}$ mol

As a conclusion, we can say that:

- microRNAs and lncRNAs play pivotal roles in stem cells regulation
- in cancer they can act oncogenic or tumor suppressive as well
- the use of nanodoses following the principle of Hormesis make them remarkable regulatory molecules with an impressive therapeutic impact.

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