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Do Somatic Cells De-differentiate/Trans-differentiate or VSELs Initiate Cancer and Explain Plasticity in Adult Tissues?

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Abstract: Cancer cells have phenotypic features resembling embryonic stem cells and thus cancer is also defined as a 'disease of differentiation', 'stem cell disease' or 'oncogeny in blocked ontogeny'. The question whether cancer occurs due to de-differentiation/reprogramming of somatic cells or arises from resident stem cells remains unanswered but has a strong tilt towards de-differentiation of somatic cells. Similarly 'plasticity' of adult stem cells into multiple lineages for regenerative medicine is also explained by de-differentiation/trans-differentiation. This concept got a strong support from the ability of somatic cells to get reprogrammed to pluripotent state *in vitro*. However, a sub-population of pluripotent stem cells possibly gives rise to induced pluripotent stem cells rather than reprogramming of somatic skin fibroblasts. Similarly, rather than de-differentiation of somatic cells, possibly a sub-population of pluripotent VSELs (that maintains life-long homeostasis by serving as a backup pool of stem cells to give rise to tissue specific progenitors') gets transformed into cancer stem cells (CSCs) and also explain plasticity of adult stem cells. VSELs express pluripotent markers (OCT-4, NANOG, SOX-2, LIN-28), survive chemotherapy and undergo asymmetric cell divisions (self renew and give rise to progenitors with huge ability to proliferate and undergo clonal expansion). Their halted differentiation at various stages due to a compromised niche may explain heterogeneity noted in tumors. Ability of VSELs to get transformed into CSCs can also explain plasticity of adult stem calls and is discussed using pancreatic, hematopoietic and gonadal (ovarian, testicular) stem cells biology.

Keywords: Cancer, stem cells, VSELs, de-differentiation, trans-differentiation, reprogramming

INTRODUCTION

It is a widely accepted fact that cancers occur by accumulation of genetic mutations. A single mutation may not lead to cancer, but two or more hits (the classic two hit hypothesis) might. Cancer initiation involves (i) a genetic mutation (usually associated with proliferation) (ii) another genetic mutation (associated with proliferation, cell attachment, a protein regulating the epigenetic status etc.) (iii) epigenetic instability (induced by another mutation, by environment-mechanical, transcription factor or cytokine/growth factor induced). However, some mutations are strongly oncogenic and thus an epigenetic change may not be required for cancer initiation. The second hypothesis to explain cancer initiation is the involvement of cancer stem cells (CSCs). Research over years suggests that CSCs express pluripotent embryonic markers, exhibit properties to self-renew, divide indefinitely, can differentiate into multiple lineages, metastasize and are also resistant to cancer therapy. Heterogeneity is one of the hallmark features of cancers arising in various organs and includes both genetic (genomic instability) and phenotypic (diverse functional properties and expression of different lineage markers) heterogeneity. Distinct sub-populations of cancer cells exist within a tumor, suggesting cells in different states of differentiation. This was nicely reviewed by Friedman-Morviniski and Verma [1]. But CSCs still remain controversial regarding their identity and source.

In a viewpoint article published in 2016 in JAMA Oncology, Gross and Emanuel [2] discussed that since the launch of 'War on Cancer' in 1972, USA government alone has spent over $100 billion on cancer research, resulting in fundamental discoveries and millions of publications. However, the actual clinical progress has remained modest with cancer mortality decreasing from about 200 to 166 deaths per 100,000 as of 2012. This almost 17% reduction has largely been attributed to 50% decreased smoking over the last 50 years. We still do not understand how cancer initiates and thus it is important to think differently.

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IDENTITY OF CANCER STEM CELLS

Studies on normal and malignant hematopoiesis led to the identification of hematopoietic (HSCs) and leukemia (LSCs) stem cells. Bonnet and Dick [3] were the first to identify LSCs by florescence activated cell sorter (FACS) from human acute myeloid leukemia (AML) which on transplantation in SCID mice initiated leukemia. These LSCs showed cell surface expression of CD34+ CD38− which is shared with immature HSCs. However, Blair et al [4, 5] showed that LSCs do not express Thy-1 and c-Kit which are specific markers on HSCs. Thus either the LSCs lose these markers or arise from more primitive stem cells to HSCs. Moreover xenograft models used to identify LSCs do not take in account the role of compromised microenvironment leading to cancer formation as the SCID mice lack an intact immune system. Thus, besides xenograft model, transgenic models have also been used to demonstrate presence of LSCs including PTEN (phosphatase and tensin homologue)-null leukemia model [6].

First study reporting CSCs in solid tumors was by Al-Hajj et al [7] who isolated LIN−/ESA+/CD44+ and CD24− cells from human breast tumor which showed tumor-initiating ability on transplantation in mammary glands of NOD-SCID mice. After this report, similar CSCs have been reported in various other solid tumors including brain cancers, prostate cancer, melanoma, multiple myeloma, colon, pancreatic, and head and neck cancers [8].

SOURCE OF CANCER STEM CELLS

It has been proposed that somatic cells probably get reprogrammed to produce CSCs due to continued inflammation, infections or by other mechanisms. Somatic cells may de-differentiate back into pluripotent CSCs *in vivo* similar to their ability to get reprogrammed into induced pluripotent stem (iPS) *in vitro* when exposed to specific 'Yamanaka factors' e.g. when transcriptional factors like Wnt signaling is strongly activated [1]. The CSCs paradigm is more complex and not yet well accepted. As pointed above, there is a disparity in marker expression on HSCs and LSCs. Besides, markers for CSCs are expected to be same in different types of breast cancers however, markers like CD24, CD44, ALDH and SOX2 do not show co-expression on a specific cell type nor localize specifically at the tumor/stroma interface as expected. Tumors could arise from 200 $ESA^{+}CD44^{+}CD24^{-/low}$ $Lineage^{-}$ T1 or 1,000 $CD44^{+}CD24^{-/low}Lineage^{-}$ T2 cells [7]. Also oncotherapy fails to enrich cells expressing these markers. Thus possibly the putative CSCs get enriched based on ability to initiate tumor formation on transplanting in xenograft mouse model but have not yet been successfully purified at a single cell level [9–11].

The intimate relationship between embryogenesis and oncogenesis is a prevailing theme in cancer biology. It is intriguing to note that various groups have reported that embryonic genes are re-expressed in cancer cells [12, 13]. Based on similarities observed between certain cancer cells and embryonic cells, Julius Cohnheim first proposed in 1875 that cancers arise from ''embryonic rests'' cells leftover from embryogenesis. There exists a growing evidence of cross-talk and correlation between stemness pathways, tumor progression and metastasis and the aberrant expression of OCT4, NANOG, and SOX2. Molecular mechanisms that regulate stem cell self-renewal in the early embryo possibly get re-activated during the dysregulated proliferation observed in tumorigenesis. However, the precise underlying mechanisms remain poorly understood. Expression of these mRNA transcripts is usually higher in tumor cells than in non-tumor tissue. Besides embryonic markers, several reports have shown ubiquitous expression of OCT-4A, FSHR, and CD133 on various types of tumors [14, 15].

Similar to CSCs (which exhibit characteristics including ability to self-renew, plasticity/transdifferentiation into multiple lineages, resistance to oncotherapy and metastasis) adult stem cells 'plasticity' has also attracted lot of discussion. A recent article published in Nature Reviews Molecular and Cellular Biology by Merrel and Stanger [16] concludes that de-differentiation/trans-differentiation explains plasticity of adult stem cells by giving examples in invertebrates, teleosts, amphibians and mammals to support the concept. If this concept is true, then both 'plasticity' and 'ability to initiate cancers' lies with somatic cells and their ability to de-differentiate/trans-differentiate. Besides de-differentiation of somatic cells, it is also possible that very small embryonic-like stem cells (VSELs) known to exist in adult organs may be responsible for cancer initiation and also 'plasticity' in adult tissues (discussed below, Figure 1). It is due to changes in the microenvironment that stem cells may become malignant and get transformed into CSCs.

It is intriguing that the mechanism of iPS cells generation is still not understood. It has recently been shown that a sub-population of SSEA3+ MUSE (multi-lineage- differentiation stress enduring) cells among skin fibroblasts possibly give rise to iPS cells [17, 18] rather than dedifferentiation/reprogramming of somatic cells to pluripotent state [19] according to the elite model rather than the stochastic model [20]. A sub-population of pluripotent stem cells expressing SSEA4+ were MACS sorted from skin fibroblast culture and used for somatic cell nuclear transfer and this resulted in enhanced development and quality of SCNT cloned embryos in vitro [21]. Since both MUSE cells reported by Dezava's group [17, 18] and VSELs reported by Ratajczak's group [22] are pluripotent, can differentiate into multiple lineages, non-tumorigenic and survive various insults, we believe that MUSE and VSELs are possibly different names given to the same stem cell (and henceforth will refer to them as VSELs). The present correspondence is to build a case in favor of VSELs as the 'source of CSCs' and also to explain 'plasticity' of adult stem cells rather than reprogramming of somatic cells to pluripotent state. A crucial observation which goes against the concept of

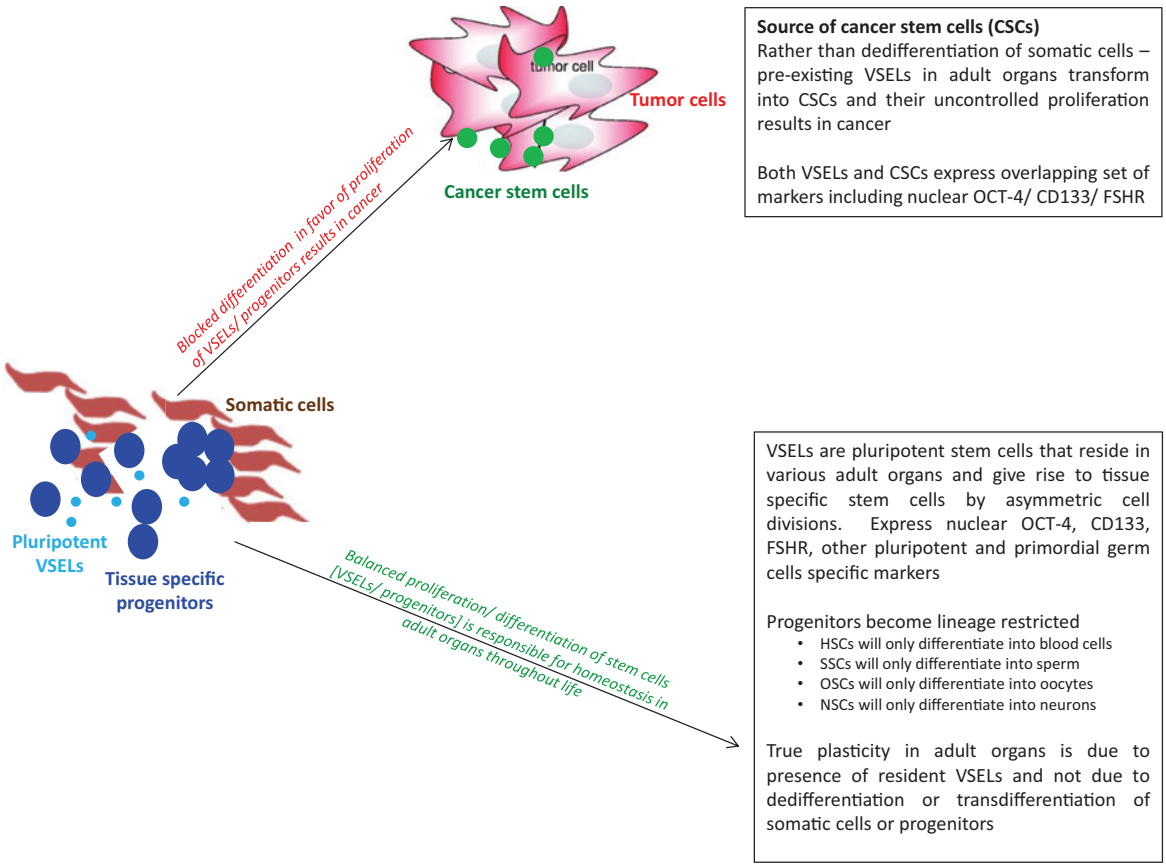

Figure 1. Schematic representation of how VSELs that exist in few numbers in various adult organs may be implicated in the observed plasticity of adult tissues and also cancer initiation.

trans-differentiation/de-differentiation during regeneration and in cancer initiation is why not all cells get reprogrammed, why is it not a global change, why does it remain very inefficient and heterogeneous?

VSELs ARE PLURIPOTENT STEM CELLS IN ADULT TISSUES

VSELs are a novel population of pluripotent stem cells that exist in all adult body organs and are an overlapping population of primordial germ cells that rather than migrating to the gonadal ridge to give rise to the germ cells, mobilize to all the developing organs and survive throughout life and serve as a backup of primitive stem cells to give rise to adult stem cells by undergoing asymmetric cell divisions and thereby maintaining life-long homeostasis. They express pluripotent embryonic-like markers, have the ability to mobilize in response to stress, self-renew (under certain conditions), differentiate into 3 germ layers and also germ cells and most importantly survive oncotherapy. These stem cells have been recently reviewed [22, 23]. They also comprise a sub-population among mesenchymal stem cells [24]. Table 1 is a compilation of various properties of cancer stem cells and various reports showing that VSELs exhibit similar properties. This strongly suggests that VSELs could possibly be the stem cells that initiate cancers and also responsible for plasticity in adult tissues. Ratajczak's group was the first to suggest that VSELs may contribute to cancerogenesis [25–27].

Cancer initiation and heterogeneity noted in tumor tissues can easily be explained by the fact that the stem cells niche gets affected to varying extent (with age, due to exposure to endocrine disruptors, continued infection/ inflammation) and as a result VSELs undergo uncontrolled proliferation, being pluripotent could explain multiple lineages in the tumor tissue and also undergo differentiation to varying extent. Similarly VSELs are a sub-population among HSCs and have the true regenerative potential. Pancreas, hematopoietic and gonadal (ovary and testis) stem cells biology is discussed below with a focus on somatic cells reprogramming versus VSELs ability to explain cancer initiation and plasticity.

PANCREATIC STEM CELLS BIOLOGY AND CANCER

Pancreas harbors α-, β- and δ-cells and has been a major focus for transdifferentiation research. Oct-4, Nanog and Sox-2 are expressed in pancreatic cancer cell lines as well as in pancreatic tumor samples [28, 29]. Wen et al [30] studied tissue microarray of human pancreas carcinoma and adjacent non-cancerous tissue and found both Oct-4 and Nanog to be strongly expressed in metaplastic ducts. Lu et al [31] showed that double knockout of Oct-4 and Nanog reduced proliferation, migration, invasion, chemo-resistance, and

Table 1. Comparing CSCs and VSELs

Cancer stem cells	Very small embryonic-like stem cells
Rapid proliferation of cancer cells but CSCs survive oncotherapy and result in recurrence	Like cancer cells, adult tissue progenitors like SSCs/OSCs/HSCs undergo rapid clonal expansion but VSELs survive various insults (Bhartiya et al, 2016; Anand et al, 2016; Patel and Bhartiya, 2016, Shaikh et al, 2016; Ratajczak et al, 2011)
Have ability to differentiate into various cell types	VSELs are pluripotent and can differentiate into various cell types
Show ability of migration and invasion	VSELs migrate to the site of injury in order to restore homeostasis (reviewed in Bhartiya et al, 2016)
Undergo asymmetric and symmetric cell divisions	VSELs (with nuclear OCT-4) undergo asymmetric cell divisions to give rise to tissue specific progenitors (with cytoplasmic OCT-4). This has been clearly demonstrated in both testis and ovary (Patel and Bhartiya, 2016; Patel et al, 2013)
Form spheres which stain positive with alkaline phosphatase	VSELs differentiate into tissue specific progenitors which in turn undergo symmetric divisions and clonal expansion (Patel and Bhartiya, 2016; Patel et al, 2013)
Expression of embryonic transcription factors OCT-4, NANOG and SOX2 / CD133/ FSHR	VSELs also express all these markers (reviewed in Bhartiya et al, 2016)

tumorigenesis of pancreatic cancer stem cells in vitro and in vivo. Gao et al [32] showed that miR-335 might inhibit progression and stem cell properties of pancreatic cancer by targeting OCT4. Lin et al [33] reported that knock-down of OCT4 suppresses the growth and invasion of pancreatic cancer cells. Thus cells expressing OCT-4, NANOG and SOX-2 could possibly be the cancer stem cells in the pancreas.

Pluripotent markers have been reported in pancreatic cancers (mentioned above) by several investigators but what is the source of these stem cells? Presence of stem cells in normal adult pancreas remains controversial. Merrel and Stanger [16] have reviewed published literature that when β-cells are destroyed in adults, α-cells cantrans-differentiate directly into functional β-cells whereas in juvenile, islets δ-cells first de-differentiate into neurogen-ein expressing progenitors and then re-differentiate into β-cells. Concepts like endo-reduplication of pre-existing islets [34] or trans-differentiation of ductal epithelial cells [35] into islets also exists. In addition, a novel population of pluripotent VSELs was first reported in pancreas in 2006 by Ratajczak's group [36]. We demonstrated involvement of OCT-4 expressing VSELs during pancreatic regeneration after partial pancreatectomy [37]. A total of $0.6 \pm 0.06\%$ of LIN−/CD45−/SCA-1+ cells comprise of VSELs in adult mouse pancreas cells which are also clearly visualized in cell smears. Two distinct size cells with nuclear and cytoplasmic OCT-4 have been observed in human pancreas [38, 39]. VSELs were mobilized from bone marrow and *en route* differentiated into OCT-4 and PDX-1 co-expressing progenitors which further differentiated into various cell types [37]. Poly-hormonal status of developing endocrine cells is well reported in fetal pancreas and also during ES cells differentiation into islets *in vitro* [40]. Possibly a common precursor stem cell (multipotent progenitor) exists for various cell types that later gets specialized and becomes mono-hormonal. Thus presence of polyhormonal cells rather than suggesting trans-differentiation, represent differentiation of pluripotent VSEL into islet cells *in vivo*. We have also discussed earlier how stem cells in the pancreas have eluded the scientific community in various studies [41]. Ratajczak's group reported that VSELs and MSCs selectively get mobilized (HSCs and EPCs are not affected) into circulation in patients with pancreatic cancer compared to normal subjects [42].

To conclude, rather than proposing de-differentiation of adult somatic cells to pluripotent state as pancreatic cancer stem cells and during regeneration – it is possible that uncontrolled proliferation of OCT-4, NANOG and SOX2 expressing VSELs result in pancreatic cancer and that these pluripotent VSELs are involved in formation of new islets throughout life.

HEMATOPOIETIC STEM CELLS BIOLOGY AND LUKEMIA

There was a lot of excitement in early 2000 when several groups reported plasticity of bone marrow cells and reviews were published with provocative titles like 'HSCs are 'pluripotent' and not just 'hematopoietic' [43, 44]! Various methods like trans-differentiation/de-differentiation, cell fusion or a sub-population of pluripotent stem cells could be responsible for the plasticity. Based on this intriguing 'plasticity' of bone marrow cells and safety of bone marrow transplantation, a large number autologus bone marrow trials to regenerate other body organs were undertaken– but have failed globally as no significant efficacy was demonstrated and a study by Nowbar et al [45] is an eye-opener. More than 600 discrepancies were found in 133 reports from 49 trials and the only 5 trials that were without any error showed no improvement of cardiac function.

Interestingly, Krause's group earlier explained plasticity of stem cells on basis of cell fusion but later reported non-hematopoietic, rare, pluripotent VSELs in the bone marrow to give rise to lung epithelial cells [46]. The reason why the autologus bone marrow trials failed to give promising results globally is because HSCs are committed hematopoietic progenitors (hence will successfully cure blood disorders) and not pluripotent (do not undergo de-differentiation/trans-differentiation or fusion to show plasticity). VSELs truly explain the pluripotent nature or 'plasticity' of bone marrow and their ability to

differentiate into HSCs [47] and all 3 lineages [48, 49] has been reported *in vitro*. They also participate in regeneration of mouse bone marrow after treating mice with 5-fluorouracil [50].

The existing controversy that LSCs do not express Thy-1 and cKit which are specific markers for HSCs (discussed above) is easily explained because LSCs are indeed Thy-1 and cKit negative VSELs which exists as a sub-population among HSCs. Oct-4 (Oct-3, Oct-3/4, and POU5F1) is a gatekeeper for early embryonic development, plays an important role in maintenance of pluripotent state, propagation of mammalian germline, marker for germ cell tumors and is also a key factor for reprogramming somatic cells to iPS cells. It is even expressed in several different kinds of cancers however, various studies failed to discriminate between Oct-4A and Oct-4B which are alternatively spliced isoforms and only nuclear Oct-4A is responsible for pluripotent state. We have earlier shown that Oct-4A is expressed by VSELs and Oct-4B by the HSCs and is gradually lost as cells further differentiate in mouse BM and human cord blood [50, 51]. Besides the presence of alternatively spliced isoforms of OCT-4 that has confused scientific community, VSELs are invariably discarded while processing samples for various experiments [23, 52]. Guo and Tang [53] carried out a very detailed analysis of bone marrow samples from patients with leukemia using RT-PCR, flow cytometry, PCR product sequencing and alignment with NCBI BLAST and DNAMAN software. They concluded that OCT4 protein is rarely detected with flow cytometry in leukemia cells. However, contrary to well established data on the presence of OCT-4 expressing VSELs in cord blood [51]; they were unable to detect Oct-4A in normal cord blood samples. Thus evidently it was the methodology of processing samples for various experiments that possibly resulted in their negative results. In contrast, Zhao et al [54] have recently reported that OCT-4A expression is significantly increased in the BM nucleated cells of patients with active leukemia compared to individuals in complete remission/chronic phase leukemia and normal controls whereas no significant difference was observed in Oct-4B expression. Thus it becomes evident that selective expansion of Oct-4A VSELs results in leukemia. OCT-4A may play an important role in the pathogenesis of leukemia and may serve as a molecular target for the development of novel diagnostic and treatment strategies in leukemia. More studies are required to further substantiate these findings.

GONADAL STEM CELLS AND GERM CELL TUMORS

Most strong evidence to support the notion that pre-existing pluripotent VSELs in normal adult somatic tissues could give rise to cancers comes from the germ cell tumors. Nuclear OCT-4A positive VSELs have been reported in normal mouse and human testes [55–57], ovary [58–61]. VSELs expressing nuclear OCT-4A co-exist with OSCs expressing cytoplasmic OCT-4B in ovary surface epithelium [62] and spontaneously give rise to oocyte-like structures in vitro [59, 61]. These VSELs survive chemotherapy [61, 63, 64] and self-renew in response to stress and undergo asymmetric cell division [57, 60] to give rise to tissue specific progenitors including spermatogonial (SSCs) and ovarian (OSCs) stem cells expressing cytoplasmic OCT-4B. Gidekel et al [65] suggested that Oct-3/4 is not only a distinctive marker for germ cell tumors, but also plays a critical role in the genesis of these tumors. Nuclear OCT-4A is also a very sensitive and specific marker for testicular germ cell tumors [66–68] and also Oct-4A positive stem cells have been reported in ovarian cancer ascites fluid [69].

It is well known that more than 90% of ovarian cancers arise in ovary surface epithelium [70], CSCs exist in ovary surface epithelium [71]. Virant-Klun's group is leading the field to show that similar VSELs observed in normal ovary are also visualized in ovarian cancer samples. Small round cells (2–4 μm) expressing NANOG, SOX2 and SSEA4 were detected in surface epithelium of ovarian cancer samples [72]. VSELs in ovary surface epithelium are best candidates that could undergo malignant transformation into CSCs [73]. The small sized CSCs in ovarian cancers divided rapidly and spontaneously formed tumor-like structures *in vitro* as well as *in vivo* [74].

Under normal circumstances, VSELs in testes and ovaries give rise to sperm and oocyte-like structures and under certain conditions get transformed into CSCs giving rise to tumors. Underlying reasons that lead to such malignant transformation of VSELs needs to be investigated further.

CONCLUSIONS

Similar VSELs expressing pluripotent markers (OCT-4, NANOG, SOX2), CD133 and FSHR exist in various adult organs, maintain homeostasis throughout life, are responsible for plasticity, have regenerative potential and possibly due to certain (yet not well understood) changes in the micro-environment (with advanced age) undergo uncontrolled proliferation to initiate cancer in various organs.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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