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Individualized precision treatment: Targeting TAM in HCC

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ABSTRACT

Tumor-associated macrophage (TAM) plays a prominent role in inflammatory microenvironment which contributes to hepatocellular carcinoma (HCC) progress by presenting M1 and M2 polarization. HCC, which is highly associated with inflammation, always leads to poor prognosis for recurrence and metastasis. TAMs with M2 polarization induce cellular proliferation, angiogenesis, epithelial-mesenchymal transition (EMT) in HCC. Furthermore, it also interacts with other immune cell clusters which leads to the resistance in targeted therapy and immunotherapy. This article reviews studies on the role of TAM to affect HCC treatment and provides insight into the potential individualized precision monotherapy or combination therapy in HCC.

1. Introduction

HCC comprises 75%–85% of primary liver cancer which is the sixth most commonly diagnosed cancer and the fourth leading cause of cancer-related death worldwide [1]. Especially in China, the infection of HBV and the explosion of aflatoxin accelerate the liver injury and lead to high prevalence rate of liver cancer. Meanwhile, the initiation and development of HCC is highly associated with the chronic inflammatory process [2]. TAM, a critical actor of the tumor-related inflammation, can be polarized to disparate functional phenotypes, as M1 (induced by IFN γ alone or with LPS) and M2 (induced by IL-4 and IL-13) macrophages [3]. Studies have shown that more infiltration of M2 TAM was related to worse clinical prognosis [4] by inducing to tumor cell proliferation, angiogenesis, metastasis and EMT [5–7]. Though it has reported that CD68 + HLA-DR + M1-like TAM may enhance motility of hepatoma tumor cells via NF–κB/FAK pathway [8]. In general, it is believed that M2 TAM plays pro-tumor role while M1 TAM exhibits anti-tumor function [9].

With the development of multi-omics and single-cell technology, we could further comprehend the dynamic process of TAM polarization and more detail of its phenotype. The classical marker of TAM is known as CD68 and CD163 [10,11], while a recent study by CyTOF identified TAM as CD14 + HLA-DR + PD-L1 + Lag-3 + in human HCC sample [12]. Furthermore, a high-dimensional research which combines the scRNA-seq and mass cytometry demonstrated that *Nos2* (iNOS) —a marker associated with classical IFNγ-activated and *Mrc1* (CD206)—a marker frequently were associated with immunosuppressive macrophages, and pseudotime plot was applied to show the continuous fate of macrophage in mice study [13].

TAM can interact with other immune cell clusters such as $CD8^+$ T cell which is converted to exhausted phenotype in tumor microenvironment. Some studies have demonstrated that this process may be linked to PD-1/PD-L1 and Tim3 signaling pathway [14,15]. In consideration of the immunosuppressive characteristics of TAM, the effect of TAM and its secretions may also regulate in immunotherapy like immune checkpoint therapy (ICT) and chimeric antigen receptor T-cell (CART) [16,17]. In addition, the utility of sorafenib, a targeted therapy drug for HCC, can be restrained by the high density of TAM [18–21]. Given the crucial correlation, systematically reviewing the mechanism of TAM may promote current researches and treatment development.

2. Polarization of TAM and the pathway activation in cell

The fate evolution of TAM which has diverse activities in HCC are respectively stimulated with M1 or M2 polarizing agents. Current conception shows that IFNγ with a TLR agonist, such as LPS lead to M1 polarization via Th1 response with tumoricidal function. On the contrary, M2 TAM which mediated Th2 response can be polarized by IL-4 and IL-13 to exert an influence on immunosuppression and promote tumor progress [3,9]. The function of IL-10 in TAM polarization remain doubt, some studies illustrate it involves TAM transform to M2 phenotype [22,23]. But some researches argue that it only plays an assistant role [24]. Most researches on macrophage polarization simply resorted to *in vitro* techniques to confirm the theory. This neglect the *in vivo* tumor microenvironment and rarely distinguish tissue resident macrophage (especially, Kupffer cells in HCC) from monocyte-derived

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macrophage. Thus, the polarization and activation of TAM could involve more stimuli agents and more complex mechanisms *in vivo*.

As for liver cancer, The TAM pool and the mechanism of recruitment can be discussed in two part (monocyte-derived and tissue-resident). It is currently known that chemokines (such as CCL2, CCL5, CCL15 and CCL20), cytokines (such as CSF-1) and the products of the complement cascade may act in monocyte-derived macrophage recruitment and migration [25–28]. KCs, a special subtype of macrophage which reside in liver tissue, are considered as the specific tumor-associated macrophages (TAMs) of HCC. Several researches provide evidence that the transition from tissue-resident macrophages to the TAM pool was triggered by Her2/Neu pathway in mammary carcinoma animal study [29,30].

2.1. CSF1/CSF1R

As for HCC *in vivo*, researches have shown that macrophages were stimulated with colony-stimulating factor 1 (CSF1). This process polarizes TAM, in details, through the CSF1R-MEK1/2-Erk1/2-c-Jun axis which leads to Allograft inflammatory factor 1 (AIF1) exclusively expressed in TAM [31]. It is also reported that AIF1 plays a critical role in M2 polarization and tumor proliferation and migration through increasing the secretions of CCL16. Consistently, CSF1R blockade inhibits tumor growth by altering the polarization of TAM in HCC [32]. CSF1 and CSF2 can respectively polarize bone marrow-derived monocytes to M1-like or M2-like phenotype. Using PLX3397, a CSF1R blockade, changes the intratumoral microenvironment and make TAM transfer to M1-like subset. This phenomenon of tumor inhibition is confirmed in HepG2 and HCCLM3(two human hepatoma cell lines).

2.2. Wnt/β-catenin

However, the polarization of TAM is also associated with crosstalk between hepatic tumor cells and macrophages via Wnt/β-catenin signaling pathway [5]. It detects that β-catenin, c-Myc and Axin2 increase in M2 TAM rather than M1 TAM. Furthermore, it illustrates that the signal pathway promotes M2 polarization via c-Myc and irrespective of M1 or M2 inducers. The ligand Wnt secreted by tumor change TAM and lead to itself development. While Combretastatin A-1 phosphate (CA1P), a microtubule inhibitor, has a function of anti-tumor by acting both on TAM and tumor cells via blocking Wnt/β-catenin pathway [33], it reports that the mechanism includes p150-AKT-GSK3β pathway as well.

2.3. Other signal pathways

Neurotensin (NTS) induced IL-8 production may polarize TAM to M2 phenotype by MAPK and NFκB pathways and promote EMT in HCC [34]. Furthermore, some studies illustrated the potential mechanism of Hypoxia environment in TAM transformation. Hypoxia-inducible factors(HIFs), including HIF1α and HIF2α, act on tumor cells through TAM in Hypoxia tumor microenvironment and lead to malignant activity of HCC [35,36]. Moreover, High-mobility group protein box1 (HMGB1) induced by hypoxia may regulate TAM to effect tumor progress and result in poor clinical prognosis [37](Fig. 1).

3. Pro-tumor role of TAM in HCC

The pro-tumor role of TAM in HCC is mediate by complex signaling pathways, which may directly act on tumor cells or be bridged by the suppression of other immune cells and mesenchymal stem cells (MSCs). TAM polarized to M2 phenotype can promote HCC progress by upregulating secretions and protein expression.

Generally, M2-polarized TAM influence HCC cells via the IL-6/STAT3 signaling pathway, and some studies show that it may be contributes to TLR4/STAT3 pathway [38,39]. Other hormone and growth

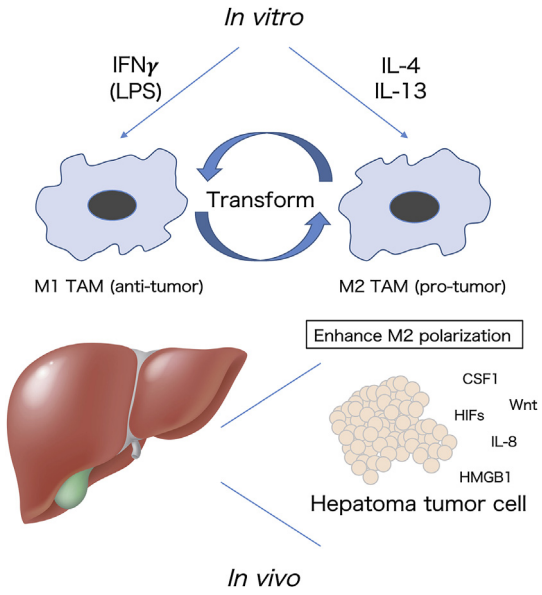

Fig. 1. TAMs differently polarize *in vitro* and *in vivo*. This process including classical factors IFNγ(LPS), IL-4, and IL-13 *in vitro*. Especially, in tumor microenvironment, more agents can promote TAM transform to M2 phenotype such as CSF1, Wnt, HIFs, IL-8, and HMGB1.

factors can also active this pathway through Janus Kinase (JAK) activation. In turn, STAT3 is phosphorylated and constitute dipolymer which mediates nucleus translocation and DNA-binding involved in proliferation and survival. Furthermore, as studies demonstrated, the crosstalk via TGFβ1 signaling pathway contributes to HCC progress too [40,41]. Interestingly, 17-estradiol (E2) could suppress tumor growth by control JAK1-STAT6 pathway which may explain the difference of morbidities in gender [42]. As mentioned in the preceding part of the text, the Wnt/β-catenin loop act as a pro-tumor factor between TAM and carcinoma too(Fig. 2).

It also reports that HCC deteriorates on account of the upregulation of protein in M2 TAM. Overexpression of oxidored nitro domain containing protein 1 (NOR1) in TAM contributes to the development of DEN-induced HCC [43]. GSF1 induce to AIF1 overexpression leading to enhanced tumor cell migration [31]. The high level of chemokine (C-X-C Motif) Ligand 8 (CXCL8) in TAM promote cancer cell proliferation and metastasis [44]. CCL2/CCR2 axis mainly attracts monocytes to residents in tissue and assists further polarization causing to promote the tumorigenesis of HCC [18]. CCL17 expressed in M2 TAM can promotes EMT and active the Wnt/β-catenin signaling in tumor cells [45]. Tumor cells activate TAM through TLR4/TRIF/NF–κB signaling pathway and upregulate the secretion of IL-1β induced by HIF1α that lead to tumor cell EMT. This may setup a relationship between hypoxia and inflammation because NF–κB and HIF1α can active each other [7]. On the contrary, receptor-interacting protein 140 (RIP140) upregulation in TAM can suppress HCC by inhibiting the NF-κB/IL-6 axis in TAM [46].

Studies also demonstrate that expression of the common MHC class I component β2-microglobulin (β2M) by cancers directly protected them from phagocytosis mediated by the inhibitory receptor LILRB1, which was upregulated in macrophages, including TAM. It also revealed that both MHC class I–LILRB1 signaling axis and CD47–SIRPα signaling axis might sensitize tumors to macrophage attack and might indirectly enhance the function of other immune cells [47].

4. Interactions among immune cells in HCC microenvironment

Immune cells and MSCs, the major composition of tumor microenvironment, educated by TAM also present immunosuppression and

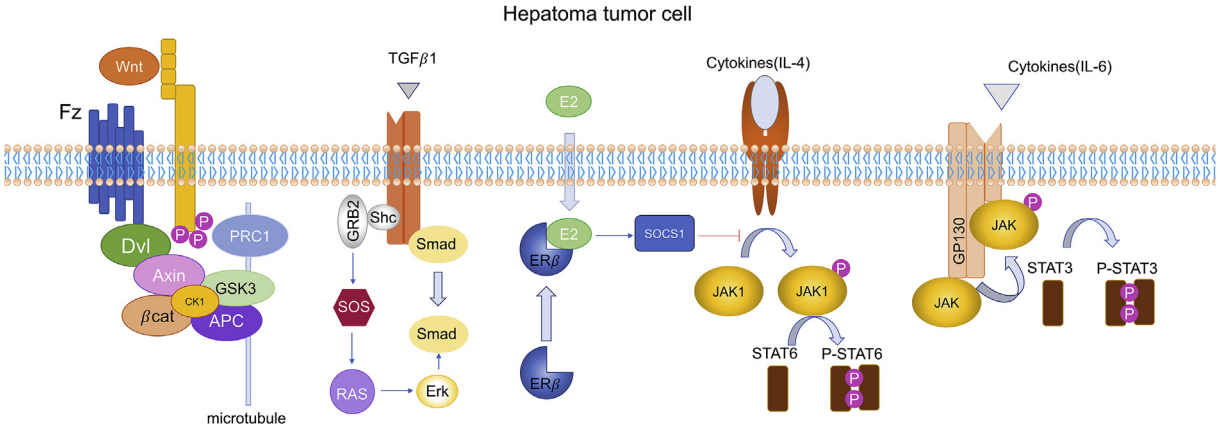

Fig. 2. Signaling pathways in hepatoma tumor cell are activated, which may be correlated with TAM. And the downstream molecular could exert an influence on enhancing the malignance of HCC.

promote the development of hepatoma tumor cells. Macrophages are essential for innate and adaptive immune response. In the condition of tumor microenvironment, M2-polarized TAM are correlated to parasite clearance, tissue remodeling, and suppress inflammation. This process may involve intricate interactions among TAM, other immune cells, and MSCs in HCC.

4.1. T cells

In sum, the infiltration of TAM is proportional to $CD4^+CD25$ + FoxP3 T cells (Tregs) and inversely proportion to $CD8^+$ T cells (cytotoxicity T cells) in HCC. It has reported that CCR2 Antagonist elevate the number of $CD8^+$ T cells by blocking the immunosuppression of TAM in liver cancer [18]. GSF1R blocking in TAM increases the infiltration of $CD8^+$ T cells, meanwhile it also decreases the infiltration of $CD4^+$ T cells [32]. The infiltration of Tregs (CCR4 +) increasing may be contributes to the higher secretion of CCL17. Furthermore, Cantharidin causes the reduction of $CD4^+CD25$ + FoxP3 T cells by change the polarization of TAM [48]. In addition to the effect on $CD4^+$ and $CD8^+$ T cells densities, TAM influence the upregulation of B7–H1 (PD-L1, CD274) in hepatoma tumor cells to suppress the function of $CD8^+$ T cells [49]. In HCC tumor micro environment, PD-L1 is mainly expressed on Kupffer cells rather than other APCs or tumor cells [14]. Conversely, $CD69^+$ T cells release IFNγ induce TAM to produce IDO and lead to pro-tumor function [50]. TAM may produce immunosuppressive cytokines (such as IL-10 and TGFβ) to affect expansion/differentiation of T cells and chemokines CCL5, CCL20, and CCL22 that recruit nTreg cells. The reduction of T cells may be correlated with the FASL/FAS pathway [26,51].

4.2. NK cells

Research also reports that IFN-γ from Non-alcoholic steatohepatitis (NASH), highly linked to inflammation and may developing to hepatocellular carcinoma, NKp46 + NK cells polarizes TAM toward M1 phenotypes. Thus absence of NKp46 + NK cells may skew toward M2 TAM and accelerate liver fibrogenesis [52]. However, high infiltration of TAM correlates with impaired functional activities of NK cells in HCC tissues. TAM may lead early activation and subsequent dysfunction of NK cells mediated by CD48/2B4 [53]. Sorafenib could modulate the crosstalk between TAMs and NK cells and promote the anti-tumor function of NK cells [54].

4.3. Other clusters

T cells and NK cells play a key role on eliminate tumor cells, however, other immune associated clusters also involve in the modulation process. Studies on interaction between B cells and TAM are limited. One research shows that CXCR3+ B cells link IL-17 inflammation to protumorigenic macrophage polarization in HCC [45]. As for MSCs, this agent can act as a poor sensor and switcher to accelerate tumor growth by recruiting and polarizing TAM [55].

5. The effect of TAM in targeted therapy and immunotherapy

In clinical, the treatment of HCC including liver resection, intra-arterial locoregional therapy (TACE and TARE alone or with sorafenib), target therapy (sorafenib, regorafenib, lenvatinib, cabozantinib, and ramucirumab) and immunotherapy such as ICT (nivolumab). The significant utility of CART was expected in solid tumor as well(Fig. 3). In consideration of the tumor heterogeneity and individual difference, precision treatment demand for more insight of systemic combination therapy. Targeting TAM in tumor microenvironment combine with the development and application of targeted therapy and immunotherapy provide a new approach.

5.1. Targeted therapy

The high density of TAM infiltration is demonstrated as a poor prognosis in HCC [4]. In another word, TAM may be one of the key factors which effect tumor recurrence and metastasis after liver resection. Sorafenib, a multi-kinase inhibitor firstly approved by FDA and widely used in clinical, increased overall survival and time to progression of patients with advanced HCC [56]. However, the utility is not significant, which may be link to the process of TAM infiltration and its effect on tumor progression, angiogenesis, and metastasis [57,58]. The dosage also should be taken into account, and it has shown that low-dose may increase the infiltration of immune cells, yet the results remain controversial. On the other hand, high-dose presents negative effects of immune microenvironment [59]. Potentially, the hypoxia induced by sorafenib can elevate the level of GSF1, HIF, and CCR4 to effect TAM lead to tumor progress [7,16,31,35,36]. PD-L1 overexpression in HCC can be including in this process as well [49]. Research has already reported that inhibiting CCR2 to block the recruitment of TAM enhances the effect of sorafenib [18]. Furthermore, studies also show that sorafenib play a role of anti-tumor through reducing the secretions of TGF- β by TAM and inhibit cancer growth, metastases, and EMT [60]. In addition, Sorafenib and gadolinium chloride (GdCl3) which suppresses macrophage-related cell markers and cytokines attenuate liver fibrosis in rats [61]. Though other anti-angiogenic agents (such as lenvatinib and cabozantinib) may also alter TAM phenotypes in tumor microenvironment [62], the data of other targeted therapy drugs is rarely reported in HCC, Further researches are required to identify the immunomodulatory function.

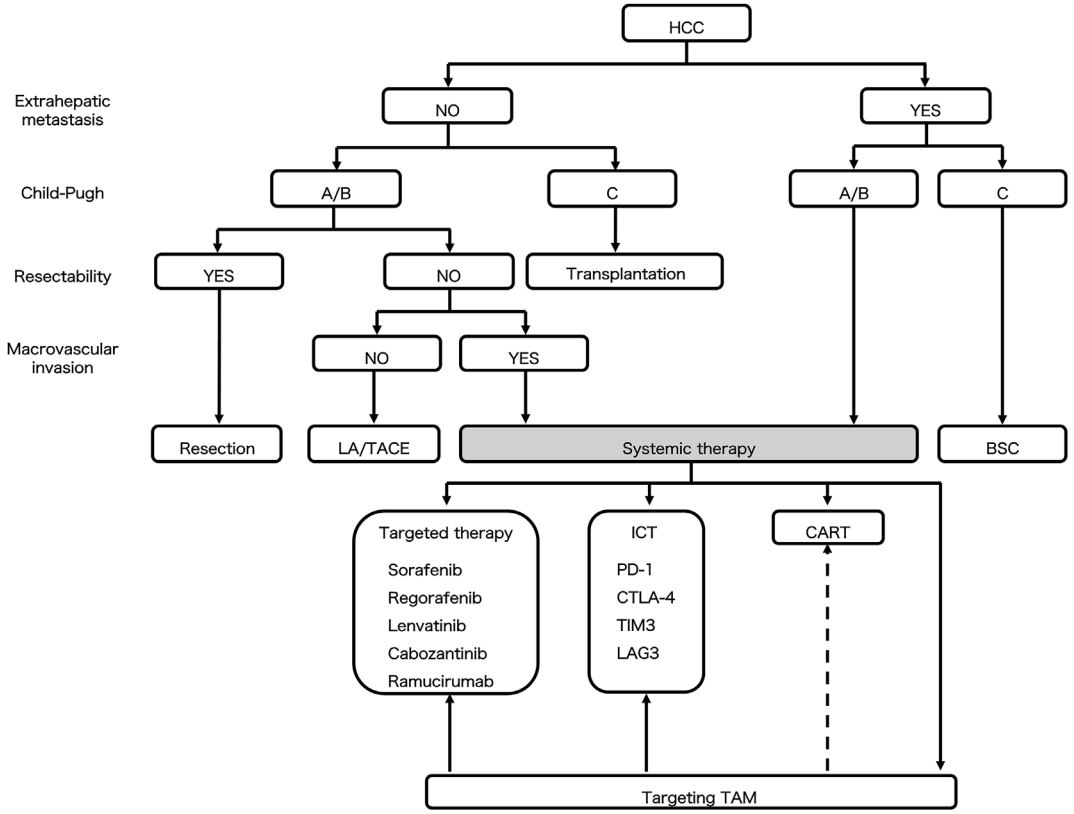

Fig. 3. Clinical guideline of HCC treatment and the strategy focus on patients with extrahepatic metastasis or unresectable tumor [44,45]. Given the interaction among targeted therapy, immunotherapy and Targeting TAM treatment, elimination of TAM may become an isolate therapy in HCC or a supplementary treatment to tumor tolerated other medicine. LA: local ablation, TACE: transcatheter arterial chemoembolization, BSC: best support care.

5.2. Immune checkpoint therapy

Given the remodeling of immune environment, immunotherapy combined with targeted therapy or resections may bring benefits to survival. Nivolumab, an anti-PD-1 antibody, is currently being investigated as a second-line therapy in a phase I/II trial. The preliminary data from its dose expansion cohort showed an objective response of 20% and a 9-month OS rate of 74% in 214 HCC patients. Current researches has reported that KCs suppression of $CD8^+$ T cells in HCC is mediated by PD-1/PD-L1 Interactions [14]. However, PD-L1 overexpression in HCC is also associated with TAM [49]. TAM also express PD-1, which correlates negatively with phagocytic potency against carcinoma, and blockade of PD-1–PD-L1 *in vivo* increases macrophage phagocytosis, reduces tumor growth and prolong the survival of mice [63]. It suggests that PD-1–PD-L1 therapies may also influence macrophages directly. Anti-PD-1 treatment can boost anti-tumor immune responses in HCC models. Interestingly, this therapy only shows efficacy with targeted drugs aiming hypoxia like CCR4 inhibitors under sorafenib treatment background [16]. Elimination of TAM provides a new approach to dealing with HCC after PD-1 therapy tolerance. Studies show that IL-17 secreted by monocytes/macrophages may suppress the function of T cell through PD-L1 in HCC too [49]. It is unclear whether the expression of PD-L1 on APC or tumor cell contributes to the efficacy of anti-PD-1/PD-L1 treatment. As to our knowledge, the relationship between the expression level of PD-1/PD-L1 on TAM and ICT responsiveness remains to be elucidated and carefully assessed [64,65]. Tim3, a T cell exhausted molecule, is expressed on TAM and contributes to poor prognosis [15]. The high upregulation of Tim3 is linked to higher tumor grades in 171 patients study [66]. Both CTLA-4 and LAG3 are important immune checkpoint as well, but CTLA4 plays a role of immunosuppression mainly via interaction between Tregs and cytotoxic T cells. While Interaction between fibrinogen-like protein 1(FGL1) and LAG3 is reported and knocking out FGL1 (upregulated in tumor cells) can decreases the density of TAM [67].

5.3. CART

Though the application of CART in primary liver cancer is rarely reported, it shows that regional infusion of CART in mice with liver metastases can delay tumor progression, but immunosuppression mediated by myeloid-derived suppressor cells in the intrahepatic space prevents complete tumor clearance [17]. Thus, further studies on combination of CART and TAM targeted therapy may solve this problem.

Nowadays, many TAM targeting drugs were approved for Clinical trials in different type of cancer (e.g. CSF-1R inhibitors and anti-bodies, CCL2 antibodies and antagonist, targeting CD47 and CD40) [68–70]. In general, TAM targeting therapeutic approaches focus on inhibiting the localization and recruitment of these cells in tumor environment and their pro-tumor functions, or reactivating their anti-tumor activities, which may become a crucial potential target in HCC treatment.

6. Conclusion

As for HCC patients, especially located in end stage with extrahepatic metastasis, we should focus on systemic therapy and immunotherapy. Individualized precision treatment demands for setting up a systemic combination therapy so that clinical performer executors do not blindly add extra drugs which may bring unnecessary side effects while achieve the most benefit to patients. Traditional resection and targeted therapy may be limited by neglecting the integrative functions of inflammation process and immune microenvironment in HCC. Further broadening the applicational range and more thorough interpretation of the mechanisms of immunotherapy may effectively

improve the therapeutic efficacy and provide the cancer patients with a solid treatment option.

Conflicts of interest

None of the authors has any potential financial conflicts of interests related to this manuscript.

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