

ITGB1BP1, a Novel Transcriptional Target of CD44-Downstream Signaling Promoting Cancer Cell Invasion

Salma M S Ahmad, Hanan Nazar, Md Mizanur Rahman, Radoslaw Stefan Rusyniak, Allal Ouhtit

Biological Sciences Program, Department of Biological and Environmental Sciences, College of Arts and Science, Qatar University, Doha, Qatar

Correspondence: Allal Ouhtit, Department of Biological and Environmental Sciences, College of Arts and Science, Qatar University, PO Box 2713, Doha, Qatar, Tel +974 4403-7572, Email aouhtit@qu.edu.qa

Abstract: Breast cancer (BC) is the most common malignancy worldwide and has a poor prognosis, because it begins in the breast and disseminates to lymph nodes and distant organs. While invading, BC cells acquire aggressive characteristics from the tumor microenvironment through several mechanisms. Thus, understanding the mechanisms underlying the process of BC cell invasion can pave the way towards the development of targeted therapeutics focused on metastasis. We have previously reported that the activation of CD44 receptor with its major ligand hyaluronan (HA) promotes BC metastasis to the liver in vivo. Next, a gene expression profiling microarray analysis was conducted to identify and validate CD44-downstream transcriptional targets mediating its pro-metastatic function from RNA samples collected from Tet CD44-induced versus control MCF7-B5 cells. We have already validated a number of novel CD44-target genes and published their underlying signaling pathways in promoting BC cell invasion. From the same microarray analysis, Integrin subunit beta 1 binding protein 1 (*ITGB1BP1*) was also identified as a potential CD44-target gene that was upregulated (2-fold) upon HA activation of CD44. This report will review the lines of evidence collected from the literature to support our hypothesis, and further discuss the possible mechanisms linking HA activation of CD44 to its novel potential transcriptional target *ITGB1BP1*.

Keywords: *ITGB1BP1*, Breast cancer, CD44, Hyaluronan, metastasis

Background

Breast cancer (BC) is the most common malignancy in women worldwide including Qatar.^{1,2} BC is a heterogeneous disease with altered biological and clinical characteristics.³ During tumor progression, cells undergo the process of epithelial-mesenchymal transition (EMT), triggering metastasis.³ Invasion is the recurring and defining event in the metastatic process,³ and elucidation of its mechanisms is critical for developing effective anti-metastatic therapies.

Invasion is a complex molecular network involving at least three major components, including cell adhesion molecules (CAMs)^{4,5} on the cell surface which facilitate the adhesion of invading cells to their surrounding extracellular matrix (ECM),⁶ proteinases that degrade the ECM, and growth factors that facilitate the growth of invading cells in a distant site. Cell adhesion maintains tissue structure and function, and changes in cell-cell and cell-matrix adhesion are of vital significance during invasion.⁵ Among the numerous CAM protein families, CD44 is the principal cell surface receptor for hyaluronic acid (HA), a major component of the ECM expressed by embryonic stem cells, connective tissue cells, bone marrow cells,^{7,8} and cancer cells.^{9,10} Binding of CD44 to HA stimulates conformational changes that triggers various oncogenic signaling pathways via various critical pathway networks (e.g., Rho GTPases, and PI3K/, AKT signaling pathways) leading to tumor cell survival, proliferation, and invasion.¹¹

To better investigate the function of the standard form of CD44 (CD44s), in BC invasion/metastasis and further elucidate its downstream signaling, we have previously developed a tetracycline (Tet)-Off-regulated expression system of CD44s both in vitro¹² and in vivo,¹³ and applied microarray analysis to identify several potential CD44s target genes.

Based on functional annotations (cytoskeletal organization and motility, ECM degradation, cell survival, and cell growth), we have classified and validated three target genes along with their signaling pathways (Cortactin, Survivin and TGF- β 2) as novel downstream target genes that underpin CD44-promoted breast tumor cell invasion.^{12,14,15}

From the same microarray data, integrin subunit beta 1 binding protein 1 (*ITGB1BP1*) was selected for further validation studies as a potential target of CD44 because of its involvement in cell motility, metastasis, and integrin binding.

ITGB1BP1, also known as ICAP-1, binds to the cytoplasmic tail of β 1 integrin.¹⁶ Specifically, it binds to the NPXY sequence motif found at the C-terminal of the β 1 integrin through its C-terminal phosphotyrosine-binding domain (PTB), which inhibits β 1 integrin interaction with the ECM.¹⁶ Under normal circumstances, *ITGB1BP1* plays a role in vascular differentiation,¹⁷ integrin activation, and focal adhesion (FA) formation.¹⁸ In this review, we collected and discussed data from the literature that support our hypothesis that *ITGB1BP1* is a potential novel target of CD44-downstream signaling underlying the process of BC cell invasion.

Structure of *ITGB1BP1*

ITGB1BP1 is encoded by a gene located on the short arm of chromosome 2 (2p25.1), which produces two isoforms; a longer isoform (*ITGB1BP1 α*), which is discussed here, and a shorter isoform (*ITGB1BP1 β*), which lacks 50 C-terminal amino acids¹⁶ and is not well-studied. *ITGB1BP1 α* has a molecular weight of 21,782 Da,¹⁶ and consists of two domains: a serine and a threonine-rich domain with a nuclear localization signal (NLS) sequence, as well as the PTB domain, which interacts with β 1 integrin.¹⁶ The availability of these domains alternates based on *ITGB1BP1*'s conformational changes, allowing exposure of either NLS sequence or the integrin binding domain.¹⁶ For instance, when β 1 integrin are overexpressed, the NLS sequence is masked, thus allowing *ITGB1BP1* binding to β 1 integrin and the localization of *ITGB1BP1* in the cytoplasm.¹⁶ In eukaryotes, *ITGB1BP1* is a phosphoprotein¹⁹ with multiple phosphorylation sites at the N-terminus as well as one site at the C-terminus.

The C-terminus features a protein kinase C phosphorylation site, while the N-terminal domain can be phosphorylated by protein kinase A (PKA), protein kinase C (PKC) and calcium/calmodulin-dependent protein kinase II (CaMKII) in order to regulate the biological activity of *ITGB1BP1*.²⁰ Site-directed mutagenesis at Thr38 has shown that phosphorylation of *ITGB1BP1* enhances cell spreading on a fibronectin matrix, while lack of phosphorylation at this site significantly inhibits cell spreading.²⁰

Functions of *ITGB1BP1*

Physiologically, *ITGB1BP1* is expressed in both normal and malignant cells. The following sections will discuss the role of *ITGB1BP1* in both normal and malignant cells.

Physiological Functions of *ITGB1BP1* in Normal Cells

ITGB1BP1 protein is present in all organs except the liver; however, *ITGB1BP1* expression varies based on the tissue and cell type.¹⁹ While inhibition of ITG- β 1 is lethal to embryogenesis, inhibition of *ITGB1BP1* is on the contrary not lethal. In fact, previous studies have shown that mice lacking *ITGB1BP1* were smaller and developed neurological disorders, bone defects,²¹ fertility defects and vascular defects.²² Moreover, *ITGB1BP1* regulates osteoblast differentiation and proliferation.²¹ *ITGB1BP1*-deficient mice displayed retardation in growth and bone mineralization, and craniofacial deformity and absence of calvaria bone development, due to reduced cell proliferation and differentiation.²¹ Similar to results from in vivo studies^{21,23,24} in vitro experiments showed impairment in cell adhesion, and migration, and organization of fibronectin matrix in *ITGB1BP1*-deficient osteoblasts.¹⁸ Furthermore, the inability of *ITGB1BP1* to interact with mutant ITG- β 1 also displayed similar abnormalities observed in *ITGB1BP1*-deficient osteoblasts, thus indicating that *ITGB1BP1* is vital for osteoblast condensation, a significant and early step during differentiation.²¹

Physiological Functions of *ITGB1BP1* in Cancer Cells

The following sections will discuss the role of *ITGB1BP1* as a regulator of the mechanisms involved in cell proliferation, adhesion, and motility, processes involved in the onset and progression of cancer.

Physiological Functions of *ITGB1BP1* in Cell Proliferation

ITGB1BP1 is known to interact specifically with the cytoplasmic domain of $\beta 1$ integrin to control cell spreading on fibronectin matrix.^{19,25} Interestingly, *ITGB1BP1* was not only observed in the cytoplasm but also in the nucleus, suggesting that it might act as a transcription factor.²⁵ The transition of *ITGB1BP1* between the nucleus and cytoplasm is $\beta 1$ integrin dependent. While upregulated $\beta 1$ -integrin expression significantly inhibited *ITGB1BP1* nuclear localization, this translocation to the nucleus is related to the stage of cell spreading on fibronectin;²⁵ this suggests a role of *ITGB1BP1* as a messenger that transmits information from integrin-dependent cell adhesion sites to the nucleus to regulate gene expression and cell proliferation.²⁵ However, the underlying mechanisms of this phenomenon are still unclear. Nonetheless, while previous in vivo studies showed deregulation of cell proliferation in *ITGB1BP1* deficient mice,²¹ overexpression of *ITGB1BP1* in the nucleus was directly proportional to an increase in cell proliferation.²⁵ Moreover, *ITGB1BP1* induced cell proliferation in a fibronectin-dependent manner, possibly through the direct or indirect activation of the c-myc promoter and interaction with nuclear factors such as Nm23-H2.²⁵ Nm23-H2 binds to a nuclease-hypersensitive element of the c-myc promoter, through which it activates *ITGB1BP1*-induced c-myc transcription and promotes cell proliferation along with upregulated cyclin D1 expression.²⁵

Previous studies have indicated that integrin $\alpha 5 \beta 1$ interacts with receptor-tyrosine kinases and activates the ERK pathway, which is critical for cell proliferation. ERK pathway activation occurs through two key mechanisms associated with integrins. Integrins, through the cytoplasmic domain of their β subunit and the transmembrane segment of their α subunit, stimulate the Src family/focal adhesion kinase (FAK) pathway and the Shc/FAK pathway, respectively.^{26–29} The α subunit-dependent pathway enhances ERK activation.²⁸ On the other hand, the β subunit-dependent pathway elongates ERK activation and promotes ERK nuclear translocation; this event is regulated by Rac.²⁸ Moreover, $\beta 1$ integrins also trigger the c-Jun NH2-terminal kinase signaling via the FAK/Cas/Rac pathway.^{30,31}

Furthermore, *ITGB1BP1* cooperates with Rho family GTPases, Rac and Cdc42, to regulate cell proliferation and cell motility.³² In fact, CD44 induced cell invasion via activation of RhoA GTPase/ROCK-1 signaling pathway.³³ As mentioned above, *ITGB1BP1* and Nm23-H2 regulate RhoA GTPase activity,²⁵ suggesting that *ITGB1BP1* and Nm23-H2 interaction can play a role in CD44-regulated tumor cell proliferation and invasion through the RhoA-GTPase pathway. CD44 is also involved in the activation of c-myc promoter; enhanced CD44 expression upregulates c-myc expression.^{34,35} In addition, CD44 also upregulates cyclin D1 by activating ERK pathway.³⁶ ERK phosphorylation, triggers extracellular and intracellular signals to promote both cell proliferation and cell migration.³⁷ The data suggests that *ITGB1BP1* is linked to CD44-downstream signaling regulating cell proliferation and adhesion.

Physiological Functions of *ITGB1BP1* in Cell Adhesion

Upon binding to their ligands, integrins merge into large clusters and recruit multiple proteins to form FAs to transduce signals to different subcellular compartments. FAs require Rho family GTPases, integrin engagement, and coordinated interaction between integrins and signaling molecules, as well as actin-binding proteins, actin microfilaments, and microtubules.^{21,38} Interestingly, Fournier et al created a double substitution of lysine for alanines in the NLS signal (KKNH)⁹ of *ITGB1BP1*, which abolished the function of NLS, subsequently leading to loss of cell adhesion.²⁵ *ITGB1BP1* protein, a negative regulator of cellular dissemination involves $\beta 1$ integrin.^{32,38} Binding of *ITGB1BP1* to $\beta 1$ integrin adversely affects the integrin's affinity for its ligand.^{21,24} Although a direct role of *ITGB1BP1* is not known in FAs,³⁸ loss of *ITGB1BP1* results in the reorganization of FA in osteoblastic, fibroblastic and endothelial cells. Talin and kindlin bind the integrin's cytoplasmic tail, and along with activated cytoskeletal and signaling proteins, they stimulate integrin binding to extracellular ligands.³⁹ The PTB-domain of *ITGB1BP1* attaches to kindlin-binding NPxY motif in $\beta 1$ integrins and displaces inhibitory proteins, thus inhibiting talin-mediated integrin activation.^{23,40} Overexpression of *ITGB1BP1* prevented talin-mediated $\beta 1$ activation, leading to FA dissociation and subsequent loss of cell adhesion.^{23,38} Moreover, *ITGB1BP1 α* , a $\beta 1A$ -integrin cytoplasmic partner, restricts the binding of both talin and kindlin to $\beta 1$ integrin, thus preventing FA assembly.²⁴ On the other hand, *ITGB1BP1* impeded ROCK1-mediated cell contractility by regulating the affinity of $\beta 1$ integrin;⁴¹ indicating transition of integrin between low and high affinity is necessary in regulating cell adhesion as well as the factors involved in maintaining an ECM environment.

Furthermore, CaMKII, a key regulator of *ITGB1BP1* α controls FA dynamics.⁴² CaMKII directly phosphorylates *ITGB1BP1* α and interrupts the intramolecular interaction between the N- and C-terminal domains of *ITGB1BP1* α ; this exposes the PTB domain allowing binding of *ITGB1BP1* α to the β 1 integrin tail and inhibits FA assembly.⁴² Overexpression of *ITGB1BP1* increases CaMKII activity and decreases the FA size.⁴² In contrast, when *ITGB1BP1* is inhibited, CaMKII does not interfere with FA assembly, suggesting that CaMKII acts on the β 1 integrin-specific adhesion sites through interaction with *ITGB1BP1*, and subsequently promoting cell migration and destabilization of FAs.⁴² The increase in cytosolic calcium levels activates CaMKII pathway and controls HA synthesis as well as various signaling pathways, including MAPK pathway.⁴³ On the other hand, increased HA synthesis promotes HA-CD44 binding, leading to the activation of various signaling pathways involved in the loss of cell-to-cell adhesion;⁹ this suggests an interaction between *ITGB1BP1* and CD44 in regulating cell adhesion.

Physiological Functions of *ITGB1BP1* in Cell Migration/Invasion

ITGB1BP1 forms a complex with ROCK-1 to promote cell migration via RhoA GTPase signaling pathway.⁴⁴ ROCK-1 binds to *ITGB1BP1* at N-terminal domain and PTB domain⁴⁴ and overexpression of *ITGB1BP1* recruits ROCK-1 allowing its translocation to the plasma membrane to form a complex with β 1 integrin.⁴⁴ RhoA induces membrane ruffles allowing its colocalization with β 1 integrin.⁴⁴ Cell migration and polarization depends mainly on the interaction between RhoA and ROCK-1,⁴⁴ suggesting that *ITGB1BP1* can enhance cell migration via activation of RhoA GTPase/ROCK pathway. Interestingly, CD44-HA interaction activates RhoA GTPase, leading to the recruitment of IP3 receptors, present in the intracellular calcium storage organelles, leading to calcium release into the cytoplasm;³⁷ this results in CaMKII activation, followed by filamin phosphorylation and subsequent induction of tumor cell migration.³⁷ Moreover, HA-CD44 binding phosphorylates myosin phosphatase and myosin light chain, leading to myosin adenosine triphosphatase activation to generate actomyosin-mediated cell migration.³⁷ ROCK also phosphorylates NHE1 resulting in the alteration of ECM, thus inducing tumor cell migration and invasion.³⁷ More interestingly, CD44 interacts with NHE1 to activate both hyaluronidase-2 and cathepsin, and promotes tumor cell invasion.⁴⁵

Cell migration can be a result of another pathway that involves ubiquitylation of *ITGB1BP1* by Smurf1 resulting in the transition from ROCK2-mediated to MRCK α -mediated cell contractility.¹⁸ In fact, HA-CD44 activates Cdc42 and phosphorylates PAK1 to form a complex with filamin and promotes cell migration and invasion.³⁷ HA-CD44 can also activate Rac1 through the recruitment of ankyrin found in the cytosol, which interacts with Tiam1, leading to cancer cell progression.³⁷ Activation of Rac1 also stimulates downstream effectors such as PAK and IQGAP1.⁴⁶ IQGAP1-Cdc42 binding mediates various signaling events to activate actin cytoskeleton and tumor cell migration and invasion.^{47–49} Furthermore, IQGAP1 complexes with ERK2 and MEK1/2 to activate ERK and MAPK signaling pathways, respectively, leading to tumor cell migration.^{50,51}

ITGB1BP1 also activates other oncogenic pathways by interacting with KRIT-1.²² KRIT-1 binds to *ITGB1BP1* through its PTB domain, competing with β 1 integrin to bind *ITGB1BP1*.⁵² Moreover, both *ITGB1BP1* and KRIT-1 promote Notch signaling pathway leading to AKT phosphorylation and activation of PI3K/AKT pathway, which subsequently promote tumor cell survival and motility.⁵³ As mentioned earlier, CD44 regulates PI3K/AKT pathway to induce tumor cell survival and motility,³⁷ suggesting a plausible association between *ITGB1BP1* and CD44. The data collected from the literature indicates that CD44 regulates *ITGB1BP1* activation via various signaling pathways involved in mediating tumor cell invasion (Figure 1). Furthermore, bioinformatics analysis revealed several transcriptional factors including NRF1, HSF and ETS2, MZF1, ELK1 that promote the transcription of *ITGB1BP1* due to the induction of PI3K/AKT and MAPK/ERK signaling pathways, respectively as shown in Figure 1.⁵⁴ In fact, and as shown in Figure 1, CD44 interacts with its ligand HA and activates several oncogenic pathways. First, CD44 activates PI3K/AKT pathway, which is also activated by *ITGB1BP1*/KRT1 complex through phosphorylation of Notch1; This leads to the transcription of *ITGB1BP1* by HSF and NRF1 transcription factors to enhance tumor cell migration and invasion. Activated PI3K/AKT pathway causes phosphorylation of Rho GEFs, which can also be activated by *ITGB1BP1*/Nm23-H2 complex. Activated Rho GEFs phosphorylate Rho GTPase, activating ROCK, and then *ITGB1BP1*, which translocate to the nucleus to form a complex with Nm23-H2 transcribing c-myc and cyclinD1, thereby enhancing tumour cell proliferation and survival. On the other hand, activated ROCK may also phosphorylate NHE1 to trigger the expression of HA through

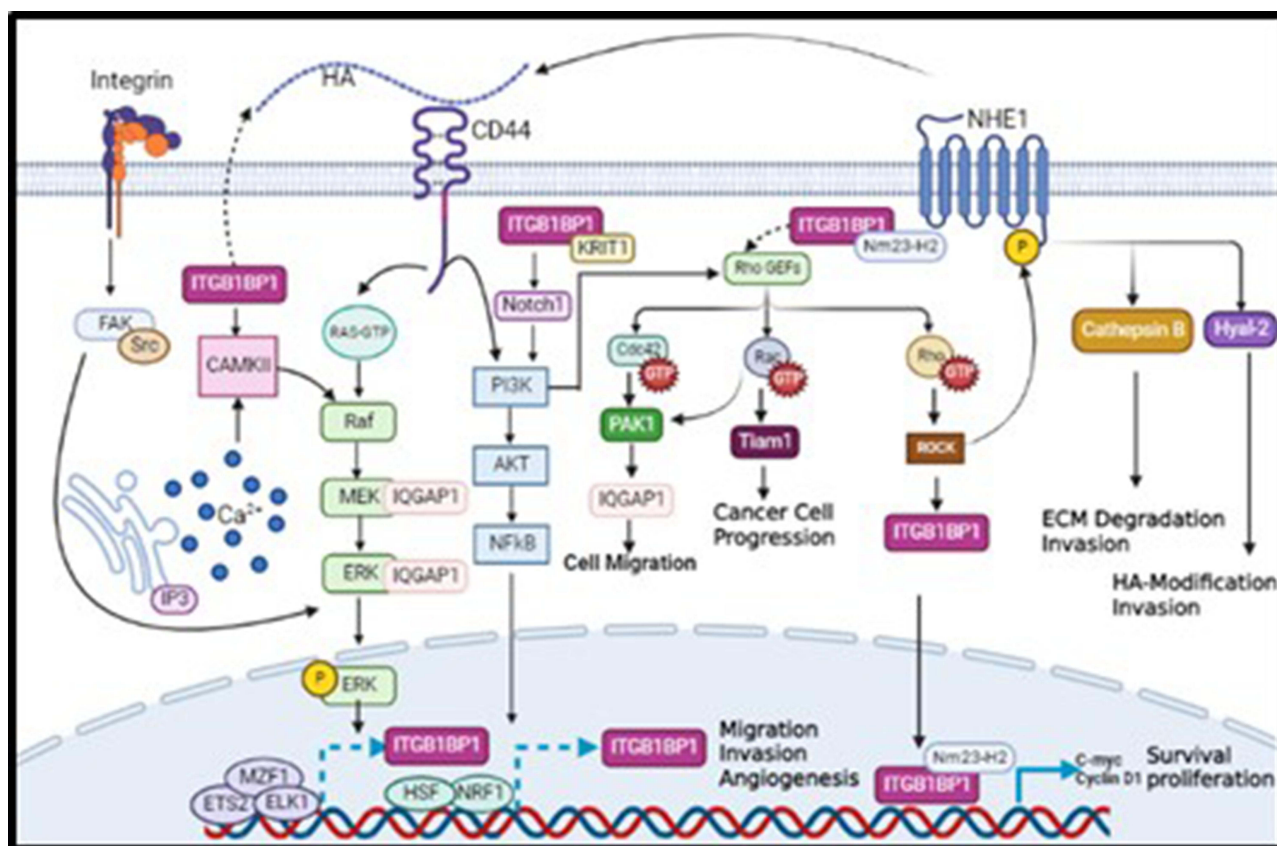


Figure 1 A proposed model describing novel molecular mechanisms linking CD44 activation by its major ligand, HA, to the transcription of its potential novel transcriptional target, *ITGB1BP1*.

Notes: Validated Signaling pathways are indicated by continued line arrows, while proposed signaling pathways are indicated by dash broken line arrows.

Hyal-2, as well as the expression of MMP9, leading to increased tumor cell invasion. In addition, activated Rho GEFs may also phosphorylate Cdc42 GTPase, activating PAK IQGAP1 leading to an increase in tumor cell migration. Interestingly, CD44 can trigger the transcription of its target *ITGB1BP1* by activating MZF1, ETS2, and ELK1 transcription factors via the MEK/ERK pathway, to promote tumor cell migration and invasion.

Conclusion

Our review has provided several lines of evidence, supporting our hypothesis that CD44-HA interaction would induce various oncogenic intermediate signaling pathways, which in turn release various transcription factors that lead to the transactivation of the CD44-target, *ITGB1BP1*. In fact, CD44 activates the transcription of *ITGB1BP1* at least via PI3K/AKT, MAPK/ERK signaling pathways, which supports our hypothesis that *ITGB1BP1* is a downstream potential novel transcriptional target of CD44/HA promoting tumor cell invasion and metastasis.

Abbreviations

AKT, Protein kinase B; BC, Breast cancer; CAM, Cell adhesion molecule; CaMKII, Calcium/calmodulin-dependent protein kinase II; Cas, CRISPR-associated proteins; CD44, Cluster of differentiation 44; Cdc42, Cell division control protein 42 homolog; ECM, Extracellular matrix; EMT, Epithelial-mesenchymal transition; ERK, Extracellular-signal-regulated kinase; FAs, Focal adhesions; FAK, Focal adhesion kinase; HA, Hyaluronic acid; ICAP-1, Integrin cytoplasmic-associated protein 1; ITG-β1, Integrin subunit beta-1; *ITGB1BP1*, Integrin Subunit Beta 1 Binding Protein 1; KRIT-1, Krev interaction trapped protein 1; NLS, Nuclear localization signal; Nm23-H2, Nucleoside diphosphate kinase B; PI3K, phosphoinositide 3-kinase; PAK, p21-activated kinases; PK, Protein kinase; PTB, Phosphotyrosine-binding domain; Rac1, Ras-related C3 botulinum toxin

substrate 1; Rho, Ras homologous; ROCK, Rho-associated protein kinase; Smurf1, Smad, ubiquitin regulatory factor 1; Tet, Tetracycline; TGF- β 2, Transforming growth factor beta 2.

Consent for Publication

Yes.

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Disclosure

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of the data; in the writing of the manuscript, or in the decision to publish the results.

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