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Review

The Essential Role of DCLK1 in Pathogenesis, Diagnostic Procedures and Prognostic Stratification of Colorectal Cancer

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Abstract. *Colorectal cancer (CRC) is the second most prevalent type of cancer among males and the third among females. CRC recurrence and poor prognosis may be related to the prevalence of chemotherapy-resistant cancer stem cells (CSCs). Recent studies have indicated the role of doublecortin-like kinase 1 (DCLK1) protein as a marker of CSC in CRC. This review focuses on the role of DCLK1 in CRC. Long-lived DCLK1-positive tuft cells can function as cancer-initiating cells. Numerous studies have shown DCLK1 overexpression to be significantly correlated with the stage of disease, the presence of metastasis and poor survival rate. DCLK1 may also be used to identify patients at high risk and those with chemotherapy-resistant tumors. DCLK1-specific drugs are examined as potential cancer treatments.*

Colorectal cancer (CRC) is the second most prevalent cancer type among males and the third among females. The 5-year relative survival rate for patients with CRC is 65% (1). The poor prognosis is related to metastasis, especially distant metastasis to the liver (2). Tumor recurrence and poor prognosis may be relevant to the prevalence of chemotherapy-resistant cancer stem cells (CSC) (3). Recent studies have shown that these cells are associated with maintaining the tumor cell population, metastatic processes and chemotherapy resistance (4). CSCs have the ability for self-renewal and may initiate tumorigenesis. Circulating CSCs have been detected in the bloodstream of patients with cancer (5, 6). There is more and more evidence emphasizing the role of specific markers for CSC detection. They may also serve as targets for cancer therapy in the future. Several CSC markers, such as leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), CD44 and progastrin peptides have been identified so far (7). The role of selected CSC markers is presented in Table I.

Recent studies have indicated the role of doublecortin-like kinase 1 (DCLK1) as a potential CSC marker in several tumor types. DCLK1 expression was shown to be correlated with low survival rate in esophageal, breast, and renal cell carcinoma (8-10). $DCLK1^+$ cells were also found in the gastrointestinal tract and were mostly expressed in the lower parts of intestinal crypt epithelium and crypt based columnar cells in normal intestine (11). The role of DCLK1 in CRC has also been highlighted. DCLK1 expression was up-regulated in CRC and was associated with CRC metastasis and poor prognosis (12). The discovery of DCLK1 may be used in the future to identify patients at high risk, monitor recurrence and evaluate response to therapy. This protein may also serve as a therapeutic target to improve outcomes. Herein, the Authors focus on the role of DCLK1 in CRC.

DCLK1 as an Important Factor in Colorectal Carcinogenesis

DCLK1 was first described in the developing brain (3). It was important in neurogenesis, cortical development and migration of neurons especially during fetal development (4, 7). This protein is a serine/threonine-protein kinase which is associated with microtubules and its overexpression leads to their elongation. $DCLK1^+$ cells were found in the health gastrointestinal tract in mice (11). Most of the $DCLK1^+$ cells

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Table I. *Significance of selected cancer stem cell markers (5, 8-12, 37, 38, 55-69).*

Marker	Protein	Description	Cancer	Clinical implications of overexpression	Future perspectives
ANXA2	Annexin A2	- Receptor for PG - Important for effects of PG on target cells	- Colon - Breast - Pancreas - Kidney - Liver	- Poor prognosis - Drug resistance	- Therapeutic target
CD44	Cluster of differentiation 44	- Transmembrane cell-surface adhesion molecule - Involved in tumor cell adhesion and invasion	- Colon - Breast - Head and neck	- Usually poor prognosis - Drug resistance	- Therapeutic target
DCLK1	Doublecortin-like kinase 1	- Protein associated with elongation of microtubules - Present in the gastrointestinal tract	- Colon - Breast - Pancreas - Kidney - Esophagus	- Poor prognosis - Drug resistance	- Therapeutic target - Detection of DCLK1+ cells in blood
LGR5	Leucine-rich repeat-containing G protein-coupled receptor 5	- Receptor for R-spondins responsible for their WNT-enhancing effects, - Target of the WNT pathway	- Colon - Nervous system	- Poor prognosis	- Therapeutic target
PG	Progastrin	- Growth factor - Ligand for ANXA2	- Colon	- Risk for developing colorectal neoplasia	- Detection of patients with risk of neoplasms

of the gastrointestinal tract belonged to the tuft cell population and were postmitotic (13). A subset of intestinal and colonic DCLK1$^+$ tuft cells was found to be long-lived, quiescent, regulated and contributed to a stem cell niche (14). Long-lived DCLK1$^+$ tuft cells can function as cancer-initiating cells (15).

Recent studies showed that the inflammatory process may also lead to induction of colorectal tumorigenesis with secondary increase of DCLK1 at the cellular level (17-19). In the early stage of CRC carcinogenesis, the expression of DCLK1 seems to increase from low-grade adenomas with worsening severity of dysplasia (18, 19). Moreover, significantly higher DCLK1 expression levels were detected in CRC tissue than in normal colonic specimens, and *DCLK1* silencing significantly inhibited cell migration, invasion, and sphere-forming potential (20). A large population of DCLK1$^+$ tumor cells was present in cancer with mutation of adenomatous-polyposis-coli (*APC*) gene particularly in CRC (21). This attribute correlated with increased pluripotency and self-renewal ability of cancer cells (21, 22). However DCLK1 expression was infrequently detected in serrated tumors of the colorectum (23).

The expression of DCLK1$^+$ CSCs was promoted by special AT-rich sequence-binding protein 2 (SATB2), RNA-binding motif containing protein 3 overexpression, *miR-15b* and lymphoid enhancer-binding factor 1 (LEF1) (24-27). Prolactin also induced DCLK1 expression in CSCs by the modulation of NOTCH signaling (28).

Studies suggested that disorders with DCLK1 expression are caused by epigenetic alterations (29-31). Hypermethylation of the *DCLK1* promoter was observed in 82% of primary CRC samples (30). The silencing of the 5'(α) promoter and transcript originating from the alternate β promoter lead to translation of an incorrect, shorter polypeptide chain of DCLK1. This alternate DCLK1 protein seems to be a response to some form of oncogenic signaling during colon carcinogenesis (31). Sarkar *et al*. discovered that transcription factor forkhead box D3 (FOXD3) strongly inhibits the activity of the β promoter, which caused the suppression of the expression of altered DCLK1 (32). Negative correlation between the levels of DCLK1 and FOXD3 was observed in pre-malignant colonic adenomas (32). In immunohistochemical examination, adenomatous polyps demonstrated higher staining for DCLK1 and significantly lower staining for FOXD3 (approximately 40% and 10%, respectively) in samples from patients who developed CRC within 15 years of polypectomy (32). Immunostaining for DCLK1 and FOXD3 was found in approximately 10% and 40%, respectively in adenomas of patients who remained disease-free in 15 years of follow-up (32). Hypermethylation of *FOXD3* was also found in human colon cancer (33). This suggests the influence of loss of FOXD3 on DCLK1 expression, with consequent colonic cancer development. Altered DCLK1 was shown to regulate prosurvival signaling, including of catenin beta 1 (CTNNB1), transcription factor p65 (RELA), prostaglandin-endoperoxide synthase 1 (PTGS1)and prostaglandin-endoperoxide synthase 2 (PTGS2) (21).

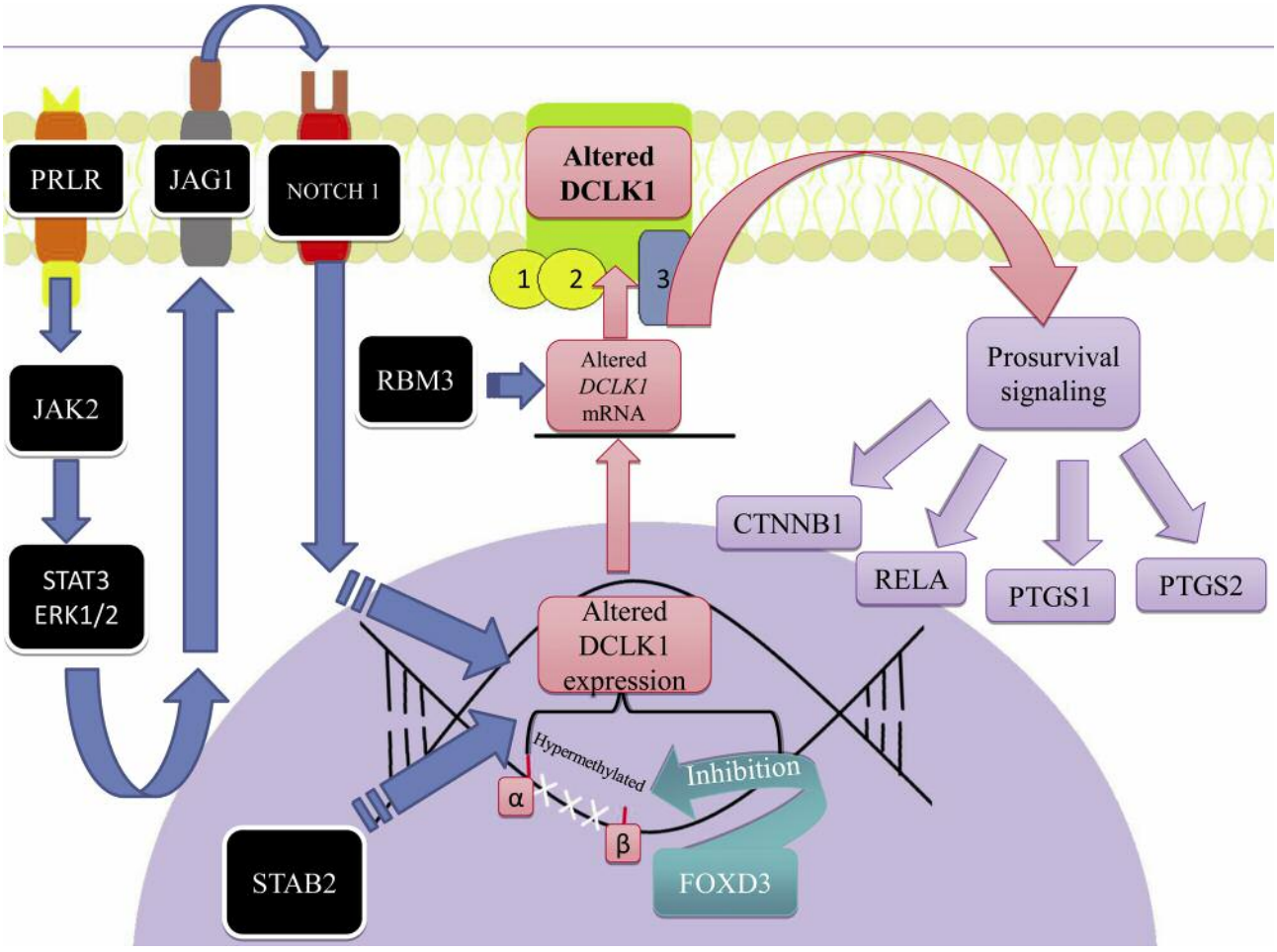

Figure 1. *Molecular pathways associated with overexpression of doublecortin-like kinase 1 (DCLK1) (22, 26-32, 70, 71). Autocrine prolactin stimulates membrane prolactin receptor of cancer cells, which causes activation of cytoplasmic factor Janus kinase 2 (JAK2). Activated JAK2 triggers downstream factors, such as extracellular signal-regulated kinases 1 and 2 (ERK1/2) or signal transducer and activator of transcription 3 (STAT3), leading to overexpression of membrane protein JAG1 in cancer cells. JAG1 is a ligand of NOTCH receptor 1 (NOTCH1). NOTCH1 receptor stimulation by other cancer cells causes excitation of DCLK1 expression. Overexpression of alternate DCLK1 can also be induced by overexpression of transcription factor stabilin-1 (STAB2) in cancer cells. Transcription of altered DCLK1 begins from the β promoter (β) because of hypermethylation of the α promoter (α). forkhead box D3 (FOXD3) can inhibit β promoter with lack of expression of altered DCLK1. In order to avoid degradation by cellular protective mechanisms, alternated mRNA of DCLK1 bind to RNA binding motif containing protein 3 (RBM3). Altered DCLK1 protein regulates prosurvival signaling including of catenin beta 1 (CTNNB1), transcription factor p65 (RELA), prostaglandin-endoperoxide synthase 1 (PTGS1) and prostaglandin-endoperoxide synthase 2 (PTGS2). PRLR: prolactin receptor; RBM3: RNA-binding motif containing protein 3; 1: doublecortin domain DCX1; 2: doublecortin domain DCX2; 3: serine/threonine kinase domain.*

Additionally, knocking down *DCLK1* reduced tumor cell stemness and progression (21). Molecular pathways associated with DCLK1 overexpression are presented in Figure 1.

The epithelial–mesenchymal transition (EMT) is a biological process that allows a polarized epithelial cell to assume a mesenchymal cell phenotype, which includes enhanced migratory capacity and invasiveness (34). EMT phenotype development among CSCs can be caused by vimentin overexpression (35). Due to an increase of β1-integrin and the loss of the junction protein E-cadherin, vimentin may mediate changes in cytoskeleton architecture (36). Studies have revealed the association between DCLK1 overexpression, an increased level of vimentin and reduced level of E-cadherin (12, 22, 37). DCLK1 may consequently affect the EMT of CSCs.

Diagnostic Opportunities in CRC Based on DCLK1

Studies have suggested that CSCs are mainly associated with the invasive properties of tumors, such as metastasis, tumor progression, recurrence and resistance to treatment. Mirzaei

Table II. *Comparison of diagnostic methods for detecting doublecortin-like kinase 1 (DCLK1) (38, 39).*

Method	Material	Target	Sensitivity (%)	Specificity (%)
(q)RT-PCR	Peripheral blood	mRNA level of *DCLK1*	81	58
ELISA	Peripheral blood	ccDCLK1	72.4	72.4

(q)RT-PCR: Quantitative real-time reverse transcription-polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay; ccDCLK1: circulating cellular DCLK1 protein.

et al. suggested it would be worth detecting CSCs in peripheral blood (PB) using specific markers (38). PB is a good source of material for investigating tumor markers because it benefits from non-invasiveness, simplicity of sampling, and accessibility (38). Mirzaei *et al*. showed overexpression of DCLK1 in PB of patients with CRC in comparison to a control group. The *DCLK1* mRNA level was also found to be significantly higher in patients with more invasive and metastatic CRC (38). Using quantitative real-time reverse transcription-polymerase chain reaction (PCR), the mRNA level of *DCLK1* as a CSC marker was assessed (30, 37, 38). The results of the research showed a significant increase in *DCLK1* mRNA level in PB of patients with CRC in comparison to controls (2.7-fold increase), with a sensitivity and specificity of 81% and 58%, respectively (38).

Another method for measuring circulating tumor cells in blood uses immunofluorescence. It is based on assessing specific markers such as DCLK1 (5). This would also be useful as a non-invasive diagnostic procedure for screening patients at high risk for developing metastatic cancer or cancer relapse (5). $DCLK1^+$ circulating CSCs were detected in the blood of patients with CRC. The number of $DCLK1^+$ cells was significantly higher in the blood of patients with CRC in comparison to that of patients scheduled for screening colonoscopy and was also correlated with tumor stage and grade (5). Using immunofluorescence, it might be possible to develop personalized treatment regimens for targeting and eliminating circulating CSCs in order to reduce the possibility of relapse or metastatic disease significantly and improve clinical results (5).

As described above, most methods are based on measurements of mRNA levels of CSC markers. However, Mirzaei *et al*. demonstrated that measurement of proteins as the final product of genes leads to more accurate results (39). They offered a method based on measuring circulating cellular DCLK1 protein using three immunoassay methods: immune-PCR, proximity ligation assay, and enzyme-linked immunosorbent assay (39). The circulating cellular DCLK1 protein level shown by all three methods was significantly increased in PB of patients with CRC compared to the control group (1.82-, 2- and 1.74-fold increases, respectively). Moreover, a higher level of circulating cellular DCLK1 protein was also correlated with tumor stage, grade and lymphatic invasion (39). Comparing these three immunoassay methods: immune-PCR with high sensitivity, proximity ligation assay with high specificity, and enzyme-linked immunosorbent assay with mid sensitivity and specificity and high reproducibility, it has been proven that the best method is an enzyme-linked immunosorbent assay because it is specific enough to evaluate DCLK1 protein expression (39). Differences between methods of detecting DCLK1 are presented in Table II. Therefore, measurement of the circulating cellular DCLK1 protein might have a diagnostic and prognostic role (39).

Another method is using quantitative methylation-specific PCR. This method is not for diagnosis, but may be useful for prognosis. This allows the assessment of hypermethylation of the DCLK1 core promoter (30). Hypermethylation of *DCLK1* was observed in 134 out of 164 analyzed CRCs and in none of 106 samples of normal colorectal mucosa, resulting in a sensitivity and specificity of 82% and 100%, respectively (30). It was noted that *DCLK1* was down-regulated in 125 analyzed CRC samples in comparison to unchanged mucosa. Subsequently, a decrease in the *DCLK1* expression of colorectal tumors was confirmed by quantitative real-time reverse transcription-PCR (30). Hypermethylation of *DCLK1* promoter is a promising new epigenetic biomarker for CRC. It has also been shown that the progeny of the CSCs, which constitutes the greater part of the tumor, have reduced expression of DCLK1 in comparison to the normal mucosa. This is very important when considering whether DCLK1-positive cells may be a weak point of CRC treatment and a promising therapeutic target (30).

The early identification of $DCLK1^+$ CSCs may allow for selection of an appropriate treatment. It is possible to achieve good therapeutic effects by directly or indirectly targeting the $DCLK1^+$ cells. Nakanishi *et al*. suggested that diphtheria toxin causes apoptosis only in the $DCLK1^+$ CSCs, but not in the $DCLK1^-$ cells (40). Interruption of delivery of the tumor progeny provided by $DCLK1^+$ CSCs can cause tumor regression (37).

Clinical Implications of DCLK1 Overexpression in Colorectal Cancer

Numerous studies have shown that DCLK1 overexpression is significantly correlated with the stage of disease, the presence of metastasis and poor survival rate (12, 31, 32, 41-43). Gao

et al. revealed the 5-year disease-specific survival in patients with low and high DCLK1 immunostaining to be 85.7% and 52.5%, respectively (12). Gagliardi *et al*. proposed a scale to show the correlation between immunoreactivity of DCLK1 in CRC and prediction of clinical outcome. This scale is based on the intensity of staining (score 0-3) combined with the percentage of positive tissue staining (score 0-3), and was shown to have an accuracy of 68.9%, a sensitivity of 64.3%, and a specificity of 69.2% (41). A score of 5 or more correlated with 60% mortality of patients in a period of 4-37 months; those below <4 correlated with 25% mortality of patients in 11-70 months (41). However, the results of Dai *et al*. suggested that patients with high DCLK1 expression exhibited a significantly longer survival than did patients with low DCLK1 expression (44). This incompatibility should be solved in a future study.

Actual molecular biology knowledge suggests the importance of FOXD3 level in CRC development. The combined expression of DCLK1 and FOXD3 exhibited significant correlation with overall patient survival, but the correlation was not any more significant that with DCLK1 alone (32). On the other hand, the expression level of FOXD3 alone did not correlate with overall patient survival (32).

Mortality in CRC arises from distant metastases (2). Gao *et al*. revealed the role of DCLK1 in the metastatic process in CRC. From among studies analyzed, two revealed correlation between DCLK1 overexpression and the possible contribution of DCLK1 to the metastatic process. However, divergence in immunoreactivity for DCLK1 in both of these studies is significant. A study performed by Gao *et al*. showed DCLK overexpression by immunohistochemistry in all cases, while another study revealed the presence of immunoreactivity for DCLK1 in only 50% of investigated cases of metastatic CRC (12, 41).

Studies showed that a high level of *DCLK1* mRNA correlated with advanced clinical stage and lymph node metastasis, but not with differentiation, location, gender, or age (12, 43). Higher *DCLK1* mRNA level was also observed in patients after neoadjuvant chemoradiotherapy (43).

Another significant type of molecule is microRNA, which is small non-coding RNA molecule. MicroRNAs consist of stable sets of small non-coding RNA that regulate complex processes during carcinogenesis, such as stemless, EMT, expression of tumor-suppressor genes and oncogenes (45-48). Weygant *et al*. investigated a 15-microRNA signature as a surrogate biomarker of DCLK1 biological activity in regard to recurrence of CRC. After approximately 75 months, the disease was found to recur in patients with a high-risk microRNA signature, corresponds high activity of DCLK1 in CRC cells (42). Low-risk microRNA signature, and thus low influence of DCLK1 on cellular pathways, was related to recurrence after approximately 150 months only in 20% of cases (42). Every patient with a high-risk microRNA signature died within 100 months (42).

Chemotherapy-resistant CSCs are responsible for unsatisfactory response to treatment (5). DCLK1-specific drugs are being examined as a potential treatment possibility in hepatocellular, pancreatic and ovarian cancer (49-53). In the study of Suehiro *et al*. the combination of 5-fluorouracil and DCLK1 inhibitor was more effective in CRC compared to individual treatment with these substances separately (54).

The latest studies have revealed new options for specific treatment pathways against CSC *via* DCLK1. Because of the role of inflammation in DCLK1-related tumorigenesis, one study reported that the inhibition of the inflammatory cysteinyl leukotriene receptor 1 (CysLT1R) through its antagonist, montelukast, is beneficial in minimizing stemness in colorectal tissue, with a reduction in tumor size in association with reduced levels of DCLK1 (55).

Furthermore, Qiao *et al*. developed a nondrug delivery system by conjugating hyaluronic acid and grafting DCLK1 monoclonal antibody to the surface of poly(ethylene glycol)-poly(D,L-lactide-co-glycolide) nanoparticles that is useful for targeting CSCs (56)

The LEF1/DCLK1 axis can be disrupted by niclosamide, which would impair the tumor-initiating and survival potential of CSCs (25). Another study revealed that siRNA-mediated specific blockade of *DCLK1* translation resulted in tumor growth arrest, corresponding with reduced expression of the oncogene *MYC* (57). The study of Osman *et al*. in addition suggested that Wnt family member 5A (WNT5A) agonist FOXY5 may complete the traditional adjuvant chemotherapy to which CSCs are resistant (58).

These findings could allow specific treatment against CSCs in CRC to be developed.

Conclusion

DCLK1 overexpression was significantly correlated with the stage of disease, the presence of metastasis and poor survival rate. Moreover, the present of DCLK1 in the PB of patients with CRC could suggest that this protein may act as a potential marker of therapy monitoring. DCLK1 may also be used to identify high-risk patients and those with chemotherapy-resistant tumors. DCLK1-specific drugs represent an innovative potential treatment for CRC.

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Conflicts of Interest

The Authors declare that they have no conflict of interest in regard to this study.

Authors' Contributions

All Authors of this work were committed to the selection of the theme and planning of article structure. Arkadiusz Gzil was responsible for research of literature about DCLK1-related molecular pathways in colorectal cancer and impact of DCLK1 on colorectal cancer carcinogesis, for formulating the section entitled 'DCLK1 as an Important Factor in Colorectal Carcinogenesis' and as a corresponding author for article submission. Łukasz Szylber revised the article draft by adding intellectual insights and provided critical advice, language correction and preparation of Figure 1. Damian Jaworski was responsible for research of literature about clinical data with regard to DCLK1 and DCLK1-target therapy by colorectal cancer, for formulating the section entitled 'Clinical Implications of DCLK1 Overexpression in Colorectal Cancer'. Joanna Dominiak was responsible for research of literature about diagnostic opportunities with regard to DCLK1 in colorectal cancer, for formulating the section entitled 'Diagnostic Opportunities in CRC Based on DCLK1' and preparing of Table II. Izabela Zarębska was responsible for research of literature about general information about colorectal cancer, cancer stem cells and role of DCLK1 in other neoplasm, for formulating the Introduction and preparing of Table I. Dariusz Grzanka revised the article draft by adding intellectual insights and provided critical advice, determining the value of work and control of progress of article preparation.
All Authors gave approval of the final version for submission.

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