

Review

Hypoxia-induced Factor-1 α in endometrial carcinoma: a mini-review of current evidence

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Summary. Despite the well-established role of hypoxia in cancer biology, the literature on its effects on endometrial cancer is scarce; it mainly refers to experimental settings rather than patient-derived results. Herein, an overview of the hypoxia inducible factor 1 α (HIF-1 α) biology, focusing on endometrial cancer, is presented. The molecular mechanisms possibly involved in endometrial cancer progression are presented, followed by a systematic approach to the current literature on immunohistochemistry evaluation of HIF-1 α expression in endometrial carcinoma. Since no consensus has been made regarding HIF-1 α evaluation, the evidence of possible involvement of HIF-1 α in endometrial carcinoma prognosis is weak. After a consensus has been made, properly powered studies may be able to clarify whether HIF-1 α can act as a prognosticator in endometrial carcinoma.

Key words: Endometrial cancer, Hypoxia, HIF-1 α , Prognosis

Introduction

Endometrial cancer is a common gynecological malignancy. It has been reported that in 2011 in the U.S.A., 46470 women are expected to be diagnosed with endometrial cancer, while 8120 women are expected to die of the disease (ACS, 2011). Endometrial cancer is usually diagnosed as being confined to the uterine corpus (NCI-USA, 2011a). However, depending on histological grade and myoinvasion depth, there is a

substantial possibility for the tumor either to recur locally, or to have already advanced to pelvic and/or para-aortic lymph nodes (NCI-USA, 2011b). Such likelihoods, requiring both modification of the initial management plan and adjuvant treatment, certainly affect prognosis (NCI-USA, 2011b). In this view research groups worldwide are investigating molecular markers as potential prognosticators of the disease, aiming to categorize patients as being of low or high risk for further treatment.

In the last decade, hypoxia (the condition of low oxygen content in the tissues) has been in the front line of cancer pathophysiology, as it has been proved to interact with several molecular pathways that regulate cancer cellular functions. The massive impact of hypoxia on cellular biology has been successfully presented by the concept of the "hypoxia signature" which consists of a hypoxia-regulated gene cluster (Chi et al., 2006). Although extensively studied in breast, ovarian, prostate, renal and other types of cancer, few reports exist on the role of hypoxia in endometrial cancer.

Herein, a mini-review of the current evidence on the role of Hypoxia-inducible factor 1 (HIF-1) and especially its subunit HIF-1 α in endometrial cancer is presented. The role of HIF-1 α is presented along with the mechanisms by which it is involved in endometrial carcinogenesis and tumor progression. Moreover, the current evidence, regarding possible HIF-1 α involvement in prognosis, is presented.

Tumor hypoxia

Tumor growth is a process which demands a constant supply of nutrients and oxygen in order to be maintained. This supply is mainly achieved via angiogenesis (Folkman, 2002); new capillaries are formed as a result of tumor and stromal cell induction,

allowing tumors both to grow and disseminate. Oxygen can diffuse at a distance of 100-180 μm from a capillary vessel (Powis and Kirkpatrick, 2004). Often, however, tumor growth outruns the existing supply rate, a fact that leads to the development of hypoxic areas - being further away from blood supplying vessels - acidosis and nutrient starvation. Hypoxia may be identified in the periphery of tumors (near the growth front) as a result of an increased tumor cell proliferation rate. Alternatively, it can be seen within tumors, since the intratumoral vascular network is relatively sparse and in general insufficient to meet the energy needs of the overgrowing tumor volume (Semenza, 2008). This is one of the main reasons for rapidly expanding tumors to be presented with foci of necrosis at the time of pathology evaluation and diagnosis.

Hypoxia is a condition of stress for the tumor itself. In response, tumor cells and their corresponding environment are challenged to express regulatory factors capable of protecting from the consequences of energy depletion, in this case apoptosis or necrosis. These factors are considered to be the commonest indirect way of identifying hypoxia in tissues (Seeber et al., 2011). Of note is that hypoxia can be directly measured by intervention techniques, measuring intratumoral PO₂ (Seeber et al., 2011); such an approach though, is restricted to experimental settings.

The most studied hypoxia-related factors are the hypoxia inducible factors (HIFs) (Semenza, 2007). Due to the HIF-related modification of the tumor microenvironment, tumor cells become independent to external regulatory stimuli, and biologically adjust to create the biochemical scheme that will allow them to migrate/metastasize to less hypoxic areas in the body (Ruan et al., 2009). In this way, HIF expression has been related to resistance to chemotherapy and radiotherapy (DeClerck and Elble, 2010; Seeber et al., 2010b), with HIF-1 being a candidate biological prognosticator for tumor cell response to treatment. Additionally, HIFs have become targets of molecular therapy, on both an in vitro and an in vivo basis (several molecules are under early clinical phase trials) (Seeber et al., 2010b).

The role of HIF-1 in hypoxia

The most studied HIF is Hypoxia-Inducible Factor 1 (HIF-1). It has been demonstrated that HIF-1 plays a crucial role in regulating the cell response to hypoxia, not only in tumor biology, but also in developmental and cardiovascular pathophysiology as well (Hoenig et al., 2008; Trollmann and Gassmann, 2009). Apart from hypoxia, HIF-1 intracellular concentrations can be regulated by other independent conditions.

Hypoxia-induced HIF-1 regulation

HIF-1 is assembled by two units named HIF-1 α and HIF-1 β . Both units are needed at a molecular ratio 1:1 in order for a functional HIF-1 to be assembled (Ruan et

al., 2009). While HIF-1 β is constantly expressed, HIF-1 α intracellular concentrations vary as a result of hypoxic conditions (Ruan et al., 2009). In normoxic conditions, HIF-1 α is hydroxylated in its oxygen-dependent degradation domain by prolyl hydroxylases (PHD) (Ruan et al., 2009; Seeber et al., 2011). The hydroxylated HIF-1 α is then recognized by a protein complex (in which the Von Hippel Lindau (VHL) protein participates) and becomes poly-ubiquitinated, a molecular modification that makes HIF-1 α susceptible to proteolysis in proteosomes (Ruan et al., 2009; Seeber et al., 2011). Additionally, the oxygen-dependent asparaginyl hydroxylase factor inhibiting HIF-1 (FIH) interferes to the process by repressing the HIF-1 pathway (Lisy and Peet, 2008). On the contrary, in hypoxic conditions, PHDs are unable to hydroxylate HIF-1 α , allowing it to escape from VHL recognition and degradation (Ruan et al., 2009; Seeber et al., 2011). At the same time FIH, due to hypoxia, is inactivated (Ruan et al., 2009). Thus, the abundant HIF-1 α binds to HIF-1 β , assembling the active HIF-1. HIF-1 then acts like a transcription factor binding to specific HIF-1 response elements (HRE) located on genes' promoters, inducing gene transcription (Ruan et al., 2009).

There is a plethora of genes activated as a result of HIF-1 activation. Most of them are part of a homeostatic mechanism supporting cell survival under hypoxic conditions. These genes may be involved in: a) pH regulation (carbonic unhydrase 9), b) glucose transportation (SLC2A transporters), c) glucose metabolism supporting glycolysis rather than oxidative phosphorylation (phosphofructokinase, glycogen synthase, lactate dehydrogenase A, phosphoglycerate kinase), d) angiogenesis (VEGF and others), e) nitric oxide synthesis (eNOS), f) erythropoiesis (erythropoietin) (Chi et al., 2006; Ruan et al., 2009; Seeber et al., 2011; Semenza, 2011). Interestingly, HIF-1 inhibits cell cycle progression by inducing cell cycle inhibitors (p21^{WAF1} and p27^{kip}) (Koshiji and Huang, 2004; Hackenbeck et al., 2009) or by inhibiting proliferation-related cell cycle regulators (cyclin D1 and others) (Chi et al., 2006; Lim et al., 2008). At the same time, HIF-1 can regulate apoptosis (induction of BNIP3 and NIX) (Ruan et al., 2009). It seems that HIF-1 regulates the cell cycle with the aim of preserving energy, thus allowing the cell to survive the hypoxic conditions. Moreover, HIF-1 has been demonstrated to induce cytokine secretion and immunosuppressive agents by tumor-associated macrophages (Lewis and Murdoch, 2005), regulating tumor immuno-escape via modification of local T-cell immune responses (Ruan et al., 2009). Finally, HIF-1 is supported as a facilitator of tumor cell invasion and metastasis (Gort et al., 2008).

Non-hypoxic HIF-1 regulation.

Apart from hypoxia, HIF-1 α stabilization has also been reported in oxygen tension-independent conditions. Such conditions are mainly genetic alterations that either

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affect the HIF-1 α degradation process or affect collateral pathways which interact with HIF-1 α . It has been shown that viral oncogenes can maintain HIF-1 α concentrations (Nakamura et al., 2009). Additionally, PHD or VHL mutations could deregulate HIF-1 α degradation and thus sustain HIF-1 related transcription (Seeber et al., 2011). An example of constitutive HIF-1 activated transcription is Von Hippel Lindau disease, caused by a loss of a VHL allele. After function has been lost to the normal allele as well, constitutive HIF-1 transcription - independent of hypoxia - is observed (Barontini and Dahia, 2010). This could be one explanation for the multiple neoplasms developed in VHL disease (Barontini and Dahia, 2010). Finally, several pathways (like EGFR, Ras, mTOR and the PI-3K/Akt pathway) interact with HIF-1, stabilizing HIF-1 α (O'Donnell et al., 2006).

The HIF-1 activation pathways, along with the relevant induced responses are presented in figure 1.

The role of HIF-1 α in endometrial physiology

Although initially the role of HIF-1 α in the human menstrual cycle was not considered significant (Zhang and Salamonsen, 2002), later on HIF-1 α was involved as a contributor of endometrial tissue repair. HIF-1 α has been found to be expressed mainly during the secretory phase and menstruation with an increasing mode,

presenting a maximum as progesterone levels fall during late secretory phase and menstruation (Critchley et al., 2006). Recently, it was shown that progesterone withdrawal, hypoxia and prostaglandins induce VEGF and IL-8 in a HIF-1 α -dependent manner (Maybin et al., 2011a,b); these factors are considered essential for endometrial tissue repair during menstruation.

HIF-1 α is a co-activator of estrogen-induced VEGF production. Inhibition of PI3K activity, in an animal model, has been reported to block both HIF-1 α activation and estrogen receptor recruitment to the VEGF promoter (Kazi and Koos, 2007; Kazi et al., 2009). Additionally, it has been shown that estrogen-induced VEGF production can happen only in the presence of HIF-1 α , implying that this procedure is hypoxia-related (Kazi and Koos, 2007; Kazi et al., 2009). According to the authors of these reports, this imperative demand for HIF-1 α presence can be explained either as an inability of ER α to activate VEGF promoter alone after binding to it, or alternatively, that HIF-1 α modulates ER α binding to VEGF promoter. Since both normal endometrium and endometrial carcinoma cells are estrogen-dependent and PI3K/AKT is frequently activated in endometrial cancer, it could be hypothesized that estrogen-induced HIF-1 α -mediated VEGF expression may be important in establishing angiogenesis, not only in normal endometrium, but in

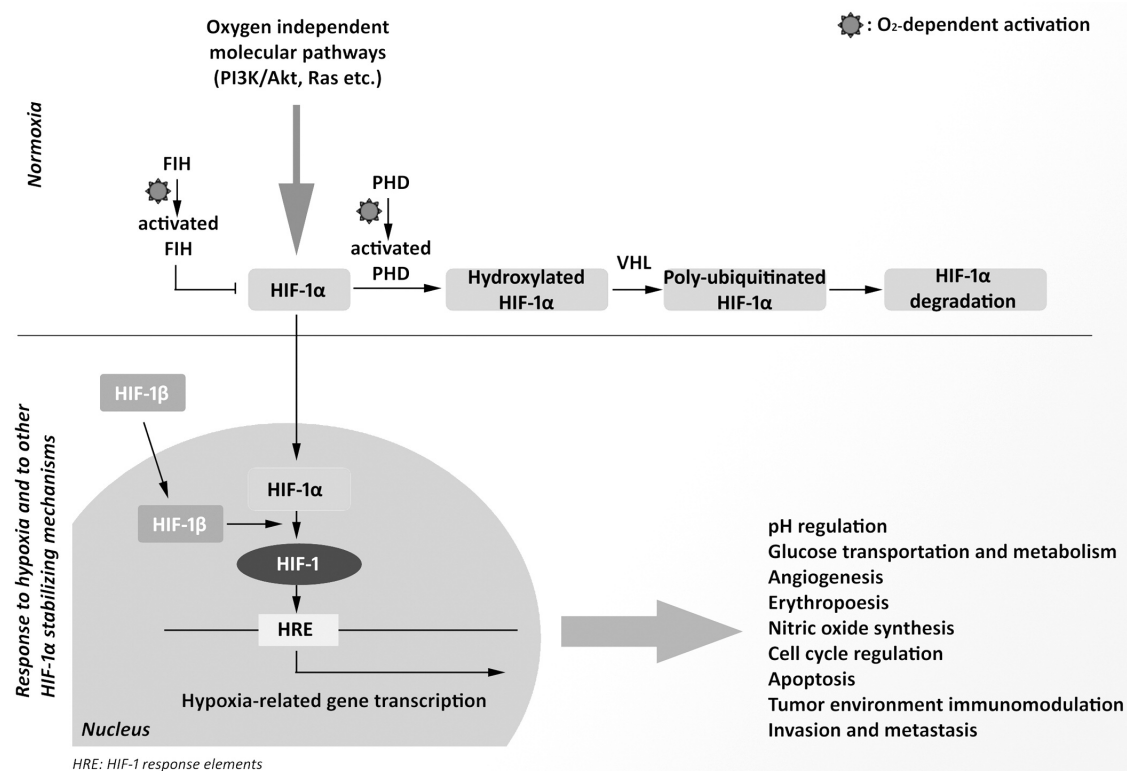


Fig. 1. Schematic presentation of the HIF-1 stabilization pathways along with the relevant hypoxia-induced responses.

endometrial cancer as well.

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Molecular mechanisms

The role of HIF-1 α in endometrial cancer stems mainly from its molecular functions, reported in endometrial physiology and pathophysiology.

HIF-1 α triggers angiogenesis in endometrial cancer. It has been shown that during myometrial invasion, myofibroblasts, being positive for HIF-1 α , produce VEGF (Orimo et al., 2001). At the same time, as mentioned above, the estrogen-dependent HIF-1-mediated VEGF production may still take place not only to normal endometrium but to endometrial carcinoma cells as well.

Additionally, it was reported that hypoxia (as was identified by HIF-1 α expression) and Prostaglandin E receptor 4 pathways act in synergy to promote endometrial cancer cell proliferation and tumor growth in an animal model (Catalano et al., 2011).

HIF-1 α is also involved in p27^{kip} regulation in endometrial carcinoma progression. It has been shown that p27^{kip} expression is lost in early endometrial carcinoma (Horree et al., 2008a). Since it is a cell cycle inhibitor, it was postulated that this was a step towards increased tumor cell proliferation. As tumor size increases at a rate that outruns the metabolic demands

for sustainable tumor growth, the developing hypoxia leads to the formation of necrotic foci. Interestingly, in the perinecrotic areas, HIF-1 α has been proven to induce p27^{kip} expression, in the view of cell cycle arrest (Horree et al., 2008a). This could ensure cell survival of the HIF-1 α /p27^{kip}-expressing cells and thus further adaptation of the endometrial cancer cell clones. Despite the importance of this finding, evidence suggests that the HIF-1 α /p27^{kip} association could rather be a part of a complex network of hypoxia-induced factors. In this sense a network of factors organized as a “hypoxia signature” has been shown in breast and ovarian carcinoma, but not in endometrial cancer so far (Chi et al., 2006).

HIF-1 α gene polymorphisms could be associated with increased HIF-1 α stability, and thus a continuous HIF-1 activation. Such polymorphism has been reported in endometrial carcinoma even as a de novo mutation, involving the substitution of a proline to serine (P 582 S) (Horree et al., 2008b). This alteration is associated with increased microvascular density in endometrial cancer cases, and thus possibly with increased tumor growth; it is not clarified whether such polymorphism can contribute to the risk of endometrial carcinogenesis, since conflicting reports exist in the field (Konac et al., 2007; Horree et al., 2008b).

Finally, HIF-1 α is stabilized by PI3K/AKT pathway, since in endometrial cancer this pathway remains unregulated due to mutations often seen on the PTEN, a

Table 1. Systematic presentation of the studies, involving endometrial precancerous lesions and/or endometrial cancer, assessing HIF-1 α with immunohistochemistry (IHC).

Reference	Sample size	Hif-1 α related remarks	Significant association with adverse outcomes ¹
Espinosa et al., 2010	64	HIF-1 α is correlated to myometrial invasion	N/A
Kuiper et al., 2010 ²	51	HIF-1 α correlates with grade and low ascorbic acid content	N/A
Seeber et al., 2010a	93	Perinecrotic HIF-1 α correlates with necrosis, recurrence, metastasis and is a prognosticator for disease-free survival	Yes
Giatromanolaki et al., 2009	72	HIF-1 α correlates with deep myometrial invasion but not prognosis	No
Ozbudak et al., 2008	100	HIF-1 α is gradually expressed from early to late stage endometrial cancer	No
Horree et al., 2008b	58	P582S polymorphism is possibly related to increased tumor angiogenesis	N/A
Horree et al., 2008a	39	HIF-1 α induces p27 ^{kip} expression and cell cycle arrest in hypoxic areas of endometrial carcinoma	N/A
Giatromanolaki et al., 2008	72	HIF-1 α was not identified as an independent predictor for death events in endometrial carcinoma	No
Horree et al., 2007b	39	HIF-1 α is increasingly expressed during the transformation from the normal to the malignant endometrium	N/A
Horree et al., 2007a ²	15	HIF-1 α is highly expressed in endometrial tubal metaplasia	N/A
Koda et al., 2007 ²	60	HIF-1 α expression is correlated to leptin and leptin receptor expression in endometrial carcinoma	N/A
Pansare et al., 2007	149	HIF-1 α is significantly more expressed in type II endometrial carcinoma. However, in type I endometrial carcinoma HIF-1 α is related to myoinvasion, tumor grade, adnexal invasion and clinical stage	N/A
Pijnenborg et al., 2007	65	HIF-1 α is not an independent prognostic factor for recurrent endometrial carcinoma	No
Yunokawa et al., 2007	82 (IHC to 37)	HIF-1 α downstream molecules may have controversial correlations to endometrial carcinoma clinical stage	N/A
Fujimoto et al., 2006	60	HIF-1 α is associated with myoinvasion in stage I patients	N/A
Schimp et al., 2006 ²	147	There is no difference in HIF-1 α expression among Caucasian and African American endometrial cancer patients	N/A
Acs et al., 2004	107	HIF-1 α is not an independent prognosticator of survival or recurrence	No
Sivridis et al., 2002	81	HIF-1 α is associated with increase microvascular density and poor prognosis	Yes

Only seven studies have investigated prognosis with conflicting results: two studies support HIF-1 α as significantly associated with adverse outcomes, while five studies do not. ¹: Overall survival, Disease Free survival, Local recurrence; ²: Reference not used in the current review, N/A: Not applicable

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known regulator of PI3K/AKT (Azueta et al., 2011).

Taking into consideration all the above reported mechanisms, both in normal endometrium and in cancer, it can be concluded that endometrial HIF-1 α may induce angiogenesis and regulate the endometrial cell cycle

Histological evidence

The literature for HIF-1 α expression in endometrial carcinoma was systematically reviewed using the terms "hypoxia inducible factor AND endometrial carcinoma" OR "HIF AND endometrial carcinoma" from 1980 to 2011. Despite the increasing interest in hypoxia regarding carcinomas of different origin, the relevant literature was not extensive. The inclusion criteria were: a) evaluation of endometrial cancer prognosis based on HIF-1 α expression and b) application of immunohistochemistry.

There is much controversy about the pattern of HIF-1 α staining to be evaluated. HIF-1 α is reported to be identified both in the nucleus and the cytoplasm of tumor cells (Seeber et al., 2011); some studies consider only nuclear staining while others try to incorporate cytoplasmic reactivity in their semi-quantitative staining score. Even if there is no consensus regarding which better represents the *in vivo* state, the proposal that only nuclear reactivity (Seeber et al., 2011) is to be accounted for seems reasonable. It takes into consideration only the fraction of HIF-1 α identified as part of active HIF-1 which is found in the nucleus; the rest of the HIF-1 α is not considered to interfere with transcription and may not even be related to hypoxia (Seeber et al., 2011). In that view, evaluating cytoplasmic reactivity could possibly lead to overestimations.

The up-to-date clinico-pathological data supports that HIF-1 α is gradually expressed, from a minimum expression in the case of benign endometrium to a moderate expression in the case of endometrial hyperplasia, and a strong one in the case of endometrial carcinoma (Horree et al., 2007b). Equally important is the finding that HIF-1 α maintains a gradual increase in expression from early to advanced clinical endometrial cancer (Ozbudak et al., 2008). The same expression pattern is also followed by the immediate downstream genes (carbonic anhydrase 9 and Glut I transporters) (Horree et al., 2007b; Ozbudak et al., 2008). This gradual HIF-1 α involvement happens in parallel with a gradual increase in microvascular density, a commonly applied marker of angiogenesis (Sivridis et al., 2002; Espinosa et al., 2010), establishing a well-accepted link between HIF-1 α , angiogenesis and endometrial carcinogenesis. Additionally, significant correlations of HIF-1 α expression with an increased ratio of angiopoietin-1/angiopoietin-2 and increased IL-8 production were shown (Fujimoto et al., 2006) in endometrial carcinoma, further supporting the role of HIF-1 α in endometrial carcinoma angiogenesis.

In endometrial cancer, HIF-1 α is expressed in both type I and type II carcinomas. However, even if it is

more abundant in type II carcinomas, clinical statistically significant correlations have been made only in the group of type I carcinomas (Pansare et al., 2007). Indeed HIF-1 α expression was related to high histological grade, myometrial invasion, adnexal or vascular invasion and, finally, unfavorable clinical stage in type I carcinomas (Pansare et al., 2007). The connection of myometrial invasion with HIF-1 α expression is in line with other studies revealing that in stage I endometrial carcinomas HIF-1 α expression is significantly correlated with myometrial invasion (Fujimoto et al., 2006; Espinosa et al., 2010).

Taking into consideration the already presented interaction between HIF-1 α and p27^{kip} (Horree et al., 2008a) the same research group showed that necrosis in endometrial cancer was significantly correlated to decreased disease-free and overall survival (Seeber et al., 2010a). Additionally, perinecrotic HIF-1 α was prognostic for shorter disease-free survival and p27^{kip} expression (Seeber et al., 2010a). Although this study is in concordance with another report supporting worse prognosis in HIF-1 α expressing endometrial carcinoma cases (Sivridis et al., 2002), the opposite has also been supported in the literature (Acs et al., 2004; Pijnenborg et al., 2007; Giatromanolaki et al., 2008, 2009; Ozbudak et al., 2008) (Table 1). The studies on HIF-1 α regarding endometrial cancer prognosis are relatively heterogeneous and controversial. This stems from the fact that: a) there is no consensus on the mode of HIF-1 α evaluation (nuclear with vs. without cytoplasmic contribution to immunoreactivity scoring, as well as different scoring systems), b) different histological compartments within the tumor are studied (perinecrotic areas vs. whole tumor), c) samples are relatively small. The whole situation may become even more confusing since it was shown that HIF-1 α downstream molecules may present controversial correlations to endometrial cancer stage (Yunokawa et al., 2007).

All the above taken together support the thesis that HIF-1 α expression is associated with increased angiogenesis and myometrial invasion in endometrial cancer. Its role however in prognosis has not been clarified yet; larger series are needed to ascertain a possible independency for HIF-1 α as a prognosticator in endometrial carcinoma.

Conclusions

Hypoxia is involved in endometrial cancer, with HIF-1 and thus HIF-1 α being the most studied. Current evidence supports HIF-1 α 's participation in both endometrial carcinogenesis and tumor angiogenesis and progression. However, such data have been only partially verified in terms of prognosis, since a) there is no consensus regarding HIF-1 α immunohistochemical evaluation, b) different histological compartments within the tumor are studied and c) most of the series are relatively small. After establishing a consensus for HIF-1 α evaluation, large series studies are expected to clarify

whether HIF-1 α could be clinically used as a prognosticator in endometrial carcinoma.

Conflicts of interest. The authors declare that they have no conflicts of interest.

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