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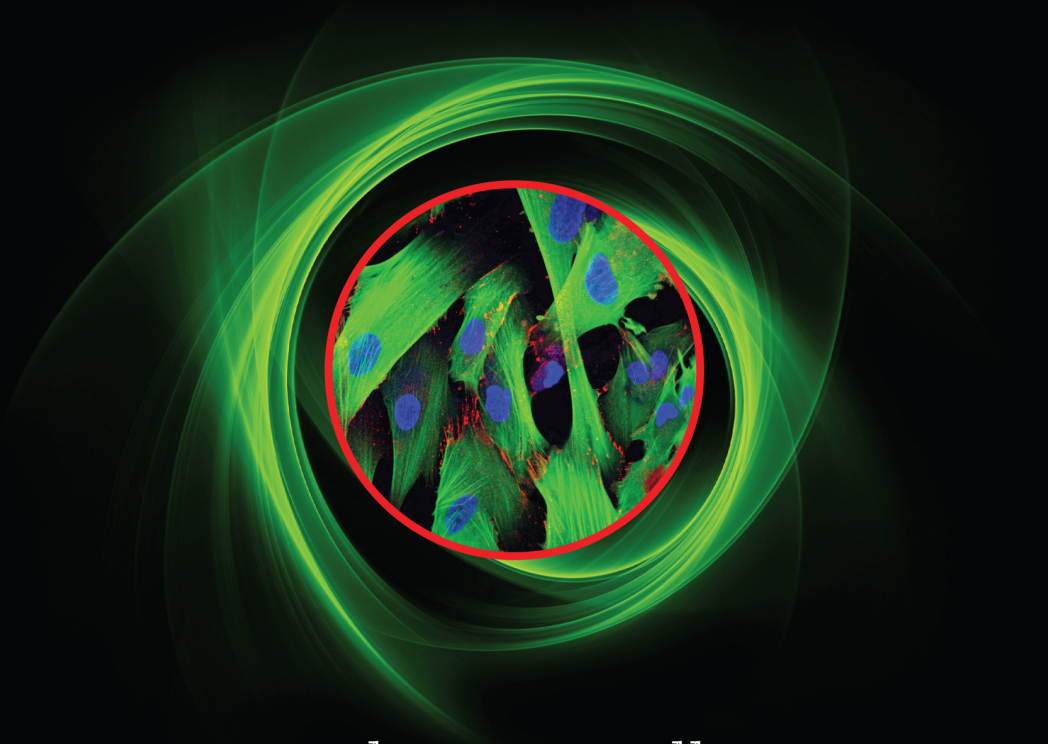
Cadherins

Types, Structure and Functions



Jonathan McWilliam
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TYPES, STRUCTURE AND FUNCTIONS

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Chapter 3

DISRUPTION OF E-CADHERIN PATTERN IN UTERINE AND MAMMARY TUMOURS

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ABSTRACT

E-cadherin (E-cadh), a member of the classic cadherins superfamily, plays an important role in epithelial cell-to-cell adhesion, encompassing the dynamic interactions between adjacent cells including the control of morphogenesis, maintenance of cell polarity and tissue architecture. Cadherins comprise a large family of cell surface glycoproteins, presenting unique extracellular regions domains known as the cadherin motifs or domains, which fold like immunoglobulin domains. They mediate strong Ca^{2+}-dependent homophilic interactions between neighbouring epithelial cells, resulting in the formation of cell adhesion "zippers." E-cadh cytoplasmic tail links to catenins and, thereby to the actin cytoskeleton and signalling proteins to form a cell-cell signalling centre: it regulates several intracellular signal transduction pathways, including Wnt/β-catenin, PI3K/Akt, Rho GTPase, and NF-KB signalling.

E-cadh plays a crucial role in the barrier formation of polarized epithelial cell layers at the interfaces contacting with the external environment, namely the uterus and the mammary gland. The maintenance of these barriers could be considered as a prime immunologic function of E-cadh, compartmentalizing potentially harmful agents away from the underlying tissue. Disruption of classical cadherin expression has been related to the occurrence of diseases driving disturbances in tissue architecture, such as inflammation and cancer.

In cancer, loss of E-cadh expression/function increases cell proliferation, cell migration, and disruption of epithelial cell homeostasis, driving cell dissociation and scattering. Alterations of E-cadh expression have also been reported during particular moments of the female reproductive physiology, namely throughout the oestrous cycle or during the embryo-maternal interaction at embryo implantation (early pregnancy). Several studies have shown the downregulation of E-cadh in malignant epithelial tumours, which has been associated with loss of cell differentiation, epithelial to mesenchymal transition (EMT) and invasion. Data also suggest that loss of E-cadh may be associated with malignant progression, metastasis, and reduced survival in multiple cancer patients.

In this chapter, we review and discuss the role of E-cadh in the uterine and mammary gland homeostasis and describe disruptive patterns of E-cadh expression in neoplastic conditions of the uterus and mammary glands in human and domestic dogs and cats.

Keywords: E-cadherin, uterus, mammary gland, carcinoma, human, dog, cat

INTRODUCTION

Cadherins are cell surface glycoproteins generally associated with cell-to-cell adhesion and recognition processes occurring in animal tissues. Cadherins form a superfamily of more than one hundred of calcium-dependent membrane proteins mediating homophilic cell-to-cell adhesion [1]. Presenting unique extracellular regions domains, known as the cadherin motifs or cadherin domains, which fold like immunoglobulin domains [2], these proteins have been found in both invertebrates and vertebrates, and in a wide array of tissues [3, 4].

Yet, classic cadherin roles go far beyond the epithelial cell-to-cell adhesion. They control cell movements underlying morphogenesis, changes in cell polarity, cell structure, and also mediate several intracellular signalling processes associated with cytoplasmic organization and motile behaviours of cells, as well as changes in gene expression to control cell differentiation and growth, and tissue architecture [5, 6]. Cadherins contribute to tissue homeostasis, participating in the tissue barrier function, cell proliferation, and migration [7]. As other type I classic cadherins, E-cadherin (E-cadh) mediates cell adhesion and is a key determinant of epithelial morphology and differentiation in most epithelial body tissues and organs [8].

E-Cadherin at the Core of Epithelial Adherens Junctions

Epithelial integrity depends on the interaction of different types of junctions, namely the tight junctions, adherens junctions and desmosomes. Together, they constitute the epithelial junctional complex. Adherens junctions (AJs) are specialized cell-to-cell adhesion sites consisting of E-cadh/catenin complexes that link to actin cytoskeleton [6], which in turn regulates the assembling, organization, stability and remodeling of AJs [9]. Connection with actin bundles allows the interaction of other types of junctions with AJs, working together to maintain the epithelial barrier. In the case of AJs, the barrier is achieved by creating a continuous adhesive

belt at the apical–lateral interface of adjacent cells. Despite providing strength and polarity to epithelial barriers, AJs present notable plasticity, which is important for tissue morphology and morphogenesis [10]. This plasticity is of upmost importance to coordinated multicellular movement or the cooperatively regulated single-cell migration [11, 12], either for embryo morphogenesis, tissue healing and tumour invasion/progression.

As said, clusters of dimeric E-cadh mediate trans-homophilic interactions between neighboring cells forming AJs [10, 13]. The plasticity of the epithelial barrier is made possible by a rapid and constant turnover of membrane E-cadh, driving its internalization through different endocytic pathways and its recycling by exocytosis [13]; in contrast, the *de novo* synthesis of E-cadh is relatively slow. The E-cadh internalization process is mediated by clathrin, which allows the fenomenon to be spacialy controled [10].

Inside the epithelial cells, the E-cadh cytoplasmic domain links to β-catenin and p120-catenin; via β-catenin, E-cadh links to α-catenin and thereby to actin [9, 13, 14]. α-catenin links to actin through either the F-actin or vinculin [13]. The binding of α-catenin to F-actin provides stability to the adhesion point, while p120-catenin stabilises the cell-to-cell adhesion by controlling the retention of E-cadh at the cell surface [9]. While linked to p120-catenin, E-cadh is guarded from clathrin-mediated endocytosis, driving the notion that p120-catenin acts as a master regulator of cadherin stability [10, 12]. The fixation of E-cadh in the cell membrane results from the inhibition of endocytosis [13]. Besides its control of cadherin internalization, p120-catenin is considered as a “set-point” for cadherin expression [15]. It is now accepted that the level of E-cadherin expression, not that of the catenins, is the rate-limiting step for the formation of E-cadh complexes and the cell adhesion [16]. It has been proposed that p120-catenin regulates the cadherin levels within the cell, and possibly the switching of the pattern of cadherin expression from one member to another, specially in cells expressing multiple cadherin types. Moreover, it has been shown that p120-catenin may shuttle to the nucleus, where it interacts with the transcription factor Kaiso, which is involved with the regulation of various cancer-related genes [17].

Depletion of extracellular calcium drives the disruption of adhesion between adjacent epithelial cells [10]. Remodelling of AJs implies the control of E-cadh internalization, and thereby the disturbance of the E-cadh/catenin complexes. The disruption of these complexes is regulated mainly by protein kinases and phosphatases that phosphorylate the AJs structural proteins [13], affecting the connection of E-cadh to the actin cytoskeleton. Small GTPases, such as the RhoGTPases, Rac and Cdc42, and corresponding effectors, have been associated to AJs remodelling [9, 10, 13]. Furthermore, it has also been proposed that Src family of non-receptors protein kinases (e.g., c-Src and c-Yes) are essential players in AJs remodelling [18]. The activation of Src kinases drives the phosphorylation of AJ componentes and the subsequente disruption of cell-to-cell adhesions, therefore contributing to cell migration.

E-Cadherin as a Key Modulator during Development and Neoplastic Progression

Cadherin endocytosis plays a significant role in physiological and pathological processes. Cadherin endocytosis is crucial in the embryo differentiation and development [5], as well as in canine trophoblast migration at implantation [19]. A similar event occurs during epithelial-mesenchymal transition (EMT), where loss of membrane E-cadh (either by cadherin endocytosis or cadherin switching) favour proliferation, migration and invasion of neoplastic cells [15, 20].

Cadherin internalization may occur through different endocytic pathways, the clathrins´ being the most studied [10, 15]. Additional pathways associated to cadherin internalization include the caveolin-mediated and macropinocytosis-like pathways. An increase in the phosphorylation of cadherin/catenin complexes drives a loss of interaction with the actin cytoskeleton, the internalization of membrane cadherin, and the loss of intercellular adhesion [21]. Nevertheless, not all the molecules entering an endocytic pathway undergo lysosomal degradation. Some cadherin molecules are recycled back to the cell membrane, allowing cells

to retain their barrier properties and their polarity. The fate of E-cadh trafficking within the cell (degradation *vs*. recycling) is influenced by a family of Src non-receptors protein kinases [16]. c-Src and c-Yes seem to have opposing roles in the outcome of the complex disassembled proteins: c-Yes drives the molecules towards the endocytic vesicles trafficking mechanism (recycling back to the membrane), whereas c-Src fosters their lysosomal degradation [16, 22]. In some tumours, Src induced down-regulation of E-cadherin depends on integrin signaling and FAK phosphorylation [23]. Increased neoplastic Src activity have been associated with tumour invasiveness [16] and the loss of membrane E-cadh expression which suppresses cell-to-cell contacts and favours cell migration, invasiveness and metastatic dissemination. Therefore, E-cadh is often used as a prognostic marker for several solid tumours [16, 24].

Dysregulation of the E-cadh complexes may drive cells into the transition to a different cell phenotype, contributing to EMT which is often associated with tumour progression and metastases. During EMT, epithelial cells lose polarity and the typical intercelular adhesion, and develop mesenchymal features together with increased migratory and invasive properties [20]. However, EMT does not occur only in cancer; it can also be observed in natural biological phenomena such as during embryogenesis, wound healing [25], or even during glandular cyclic branching (e.g, the branching of the mammary ducts) [26].

Although initial studies pointed to a reductionist binary process, EMT is now proposed as a highly coordinated plastic program, manifesting as dynamic transitional states between the epithelial and mesenchymal phenotypes [27, 28], with the identification of distinct intermediate states (epithelial, intermediate hybrid and mesenchymal states) [27, 29].

EMT is not a mandatory pre-requisite for tumour cell invasion and metastasis [23, 30]. In fact, neoplastic cells can move in a collective manner, on which depend on the disturbance of cadherin-mediated cell-to-cell contacts. This invasive behaviour is also determined by p120-catenin, and may be accomplished by either an E-cadh-dependent or independent mechanism. Down-regulation of E-cadh can be achieved by the regulation of Rac and Cdc42 activity or indirectly by inhibiting RhoGTPases and

downstream cytoskeletal dynamics involved in cell invasion and migration [31].

Cadherin switching, that alters intercellular adherence and the cadherin-associated signaling pathways, is a common event during EMT. While normal epithelial cells express particular patterns of cadherins, cells undergoing EMT start expressing different cadherin types (mesenchymal cadherins), such as Neuronal (N-) cadh, Placental (P-) Cadh, or cadherin 11, among others [20]. This switch seldom occurs at once, but follows a specific pattern, possibly responding to different internal or external stimuli. TGF-β and the Snail, Slug and Twist transcription factors have been proposed as main EMT regulators. Dysregulation of EMT may ultimately drive to cancer [25]. Cadherin switch may coordinate changes in several cell functions, namely in cell metabolism, resistance to hypoxia and programmed cell death mechanisms, and lower adhesion to extracellular matrices [32], which all contribute to proliferation and the invasive behaviour of neoplastic cells.

The loss of functional E-cadh will contribute to tumour cells resistance to apoptosis mediated by either the intrinsic or the extrinsic pathways [33-36]. More recently, Capra and Eskelinen [37] showed that the disruption of E-cadh mediated adhesion triggers the up-regulation of survivin, which suppresses apoptosis and promotes cell proliferation and migration; survivin signalling may therefore play an important role in neoplastic progression in cells not suffering EMT.

E-CADHERIN EXPRESSION IN UTERINE CANCER

E-Cadherin in the Normal Endometrium

The cadherin/catenin complex is an essencial intercellular adhesive system in the mammal endometrium, where it coordinates key morphogenetic processes, regulates epithelial differentiation and proliferation, and supports the epithelial phenotype [38]. Moreover, it also

mediates the interactions between the embryo and maternal tissues at implantation [19, 39, 40].

The few available studies on the expression of E-cadh and/or β-catenin have established the existence of cyclic variations of these molecules throughout the uterine cycle and early pregnancy in humans [41-43], sheep [44], pigs [45] and dogs [19, 46].

Albeit studies on gene expression and protein location or quantification are not always concordant, it has been proposed that progesterone determines a decline in the strength of AJs which would facilitate the trophoblast invasion across the epithelial barrier [47]. This hypothesis is supported by a decrease in E-cadh around the implantation time in ewes [44], sows [48], and dogs [19]. In the feline cyclic endometrium, a similar decrease in the intensity of E-cadh membrane labelling has been found during progesterone dominance (M.A. Pires, unpublished results). Dudley et al. [49] described a dislocalization of E-cadh from the lateral plasma membrane to the cytoplasm in uterine epithelial cells in early pregnant cats.

Furthermore, studying the canine embryo-maternal interactions at implantation Payan-Carreira et al. [19] showed that internalization of E-cadh occurs in the maternal superficial epithelial layers of the endometrium, while the invading trophoblast retain the membrane labelling. This work agrees with an involvement of E-cadh/catenin complex in the embryo implantation and further suggests that the adhesiveness strength favours the collective invasion of trophoblastic cells.

E-Cadherin in Endometrial Carcinomas

The loss of cellular polarity [50] and alterations in cell adhesion [38] are hallmarks characteristics of cancer. Multiple studies reported reduced or aberrant expression of E-cadh and/or catenins in different human epithelial cancers: thyroid and esophageal carcinoma [38], breast carcinoma [38, 51, 52], gastric and pancreatic carcinoma, bladder and prostatic carcinoma, among others [38]. Defects in the E-cadh/catenin

adhesion complex have been described in endometriosis [43] and in several gynecologic carcinomas, including ovarian, endometrial [38, 52, 53] and cervical carcinomas [38]. Endometrial cancer is the most common malignancy of the women genital tract. In the majority of cases the prognosis is good, but women with poor differentiated, deep myoinvasive tumours, or with extension of disease to other organs or lymph nodes within the pelvis, have frequently disease recurrence [54].

In endometrial cancer, E-cadh is a recognized putative marker of good prognosis [52, 55]. Loss of E-cadh expression, has been shown to drive the loss of cell adhesiveness, and contact inhibition [52], promoting tumour progression and an aggressive behaviour, invasion and metastasis in several epithelial tumours [52, 56]. In endometrial cancer, it is correlated with tumour dedifferentiation [50, 57], high grade histology [50, 54, 56], and deep myometrial invasion [50, 54, 57], higher rate of extrapelvic recurrence [54], presentation of other adverse prognostic factors and lower overall survival [52, 56]. Some studies showed that E-cadh expression is an independent prognostic factor for women endometrial carcinoma [54, 55]. Decreased membranous E-cadh expression is predictive for endometrial cancer mortality, disease progression, and extrapelvic recurrence, independent of known prognostic factors such as stage, grade, and histological subtype [54].

Feline endometrial adenocarcinomas are uncommon, poorly characterized lesions [58], that may be underdiagnosed [59], affecting even young cats [60, 61]. There are few published studies on the immunophenotype of these lesions [58] and with small case series [59-61] which makes the full characterization of these neoplasms difficult. Though, some studies reported E-cadh and β-catenin expression in both the normal feline endometrium [58], and in endometrial adenocarcinoma [58, 62]. According to Gil da Costa et al. [58], the loss of cell adhesion that occurs within these tumours does not require down-regulation of E-cadh expression; in addition, the nuclear translocation of β-catenin was not a characteristic feature of feline endometrial carcinomas.

In their study, Carico et al. [38] showed that human endometrial carcinomas may present different patterns of E-cadh and α-catenin

expression within the tumour, reflecting the intratumoral heterogeneity of the neoplastic epithelium. Similarly, feline endometrial carcinomas present a patchy pattern of the cadherin/catenin complexes within the tumour, presenting areas of membrane and areas of cytoplasmic E-cadh expression (M.A. Pires, unpublished results). It is possible that while remaining anchored to their neighbours due to E-cadh membranar expression, epithelial neoplastic cells might have an impaired ability to metastasize. This hypothesis deserves to be further explored in the case of feline endometrial carcinomas, where a benign clinical course has been frequently described [60, 63, 64].

Canine endometrial carcinomas are rare, and mostly occur in old bitches [65]. Despite both E-cadh and β-catenin expression have been reported in the normal cyclic endometrium and the early pregnant uterus [19], the available literature is sparce in studies of adhesion molecule expression on canine endometrial carcinomas. This gap on information on E-cadh and other adhesion molecules on promotion, progression and prognosis of feline and canine endometrial carcinomas shows the need of new studies on this topic to evaluate the real effect of adhesion molecules on uterus tumourigenesis.

E-CADHERIN EXPRESSION IN MAMMARY CANCER

E-Cadherin Expression in Normal Mammary Tissues

As stated above, E-cadh has been described in most epithelial tissues, including the mammary gland. In normal human breast and in canine and feline mammary gland, E-cadh is expressed by luminal epithelial cells at the cell membrane [66-69]; this pattern of expression is also observed for α- and β-catenins [70-73]. In contrast, P-cadh is restricted to the basal myoepithelial cells [74-76]. Although cadherins expression in the mammary differentiated cells is well defined and cell-type specific, their expression in progenitor or mammary stem cell populations remain unclear [26]. Basal, P-cadh expressing progenitor cells seems to be responsible for

mammary formation and branching morphogenesis, while alveolar progenitor cells most likely represent luminal cells which give rise to E-cadh positive cells [26, 77]. It has been proven that E-cadh is essential for mammary gland, after conditional E-cadh inactivation studies using knockout mouse models [26, 78].

E-Cadherin Expression in Mammary Cancer

Breast cancer is among the deadliest malignancies in developed countries [79], with the metastatic spread being the primary reason of this fatal outcome [79-81]. In canine species, spontaneous mammary tumours are the second most frequent tumour, and the most common neoplasia in the female dog. Regarding its biological behaviour, malignant cases account for up to 50% of female mammary tumours [82, 83]. In feline species, mammary neoplasias are among the most commonly diagnosed tumours in female cats [82, 84-86], accounting for 12% of all tumours and 17% of the tumours in queens [82]. Most studies reported that more than 80% of feline mammary tumours are malignant, along with rapid progression and metastasis [84, 86].

E-cadh, a putative tumour suppressor gene implicated in carcinogenesis [87, 88], is classically considered a good prognostic marker in cancer [89]; several studies proposed E-cadh as a tumour and invasion suppressor molecule, as invasion and metastasis are promoted when its expression is lost [70, 80, 90].

In human breast cancer, although representing one of the cancer types for which E-cadh has been extensively investigated for diagnostic and prognostic purposes, conclusions are inconsistent with regard to its relevance [89, 91-93]. Some studies associated low E-cadh expression with tumour size [93, 94], histological grade [93-95], distant metastasis and absence of oestrogen receptors, but not with lymph node status [94]. Although some investigators have found no association between E-cadh and the tumour stage, lymph node status or metastasis [89], other reported an association of E-cadh with lymph node status [93] and TNM stage [93].

With regard to prognosis, data is also controversial: many studies reported that E-cadh downregulation was associated with poor survival [80, 92-94, 96], but Gillett et al. [97] found that E-cadh reduction was a favorable prognostic factor, and Wang et al. [98] found no relationship between E-cadh and prognosis. These contrasting results may be associated to inter-study heterogeneity with respect to clinical data collection, immunohistochemical staining and interpretation as well as statistical modeling, that affect studies results [96].

Adhesion molecules have been extensively studied in canine mammary tumours [66-68, 70, 71, 83, 99-104]. In these tumours, E-cadh imunoexpression was first described by Restucci and coworkers [66], reporting low expression in malignant neoplasia. After, multiple studies showed reduced membranous expression of E-cadh in malignant mammary tumours, suggesting that down-regulation of adhesion molecules is a common event in canine mammary tumours [68, 71, 100]. Some studies associated low membrane expression of E-cadh and the histological type, poor differentiation, invasiveness, high proliferation and lymph node metastasis [66, 68, 71, 99, 100]. Reduction of E-cadh expression and associated catenins was also related with invasion and metastases on canine mammary tumours [66, 67, 70, 99]. These data hint a possible role of E-cadh in canine mammary tumours aggressiveness and on the emergence of an invasive and metastatic phenotype, suggesting E-cadh as a potential prognostic marker [67, 68, 71, 105]. However, although some studies found association between E-cadh and β-catenin expression and survival [68], other failed to find these associations [70, 100].

Regarding the feline mammary tumours, few studies have evaluated adhesion molecules expression and their prognostic value [106-108]. Loss of membrane E-cadh expression and its dislocation to the cytoplasm has been described in feline mammary carcinomas compared with benign tumours and hyperplastic or normal mammary tissues [106, 108, 109], as it was reported in canine mammary tumours [66, 68, 71, 100]. Albeit Penafiel-Verdu et al. [108] found an association between E-cadh expression and the grade and existence of regional metastasis at the moment of diagnosis, other studies fail to establish such associations [69,

109]. Abnormal cytoplasm E-cadh and/or catenin has been described in feline cancer cells [107-109] but not in the normal mammary tissue [107, 108], suggesting that this abnormal location might be related to a malignant transformation of feline mammary tumours [107]. Albeit not fully explored the prognostic role of E-cadh in feline mammary tumours, some studies could not establish an association between E-cadh and survival [109].

Understanding the metastatic progression of breast cancer in humans offers the opportunity to tailor individual based therapies by considering specific tumour characteristics [80]. For metastases to occur, several progressive changes are needed; these include neovascularization, decreased adherence of the tumour cells to each other, increased motility, adhesion to the extracellular matrix, and degradation of the extracellular matrix [80]. Downregulated expression of E-cadh destroys cell junctions and thus epithelial cells acquire the ability to migrate [93]. Consequently, decreased expression of E-cadh facilitates tumour invasion and metastasis [87, 88, 93]. Nevertheless, in the vast majority of human breast cancers, E-cadh expression is not lost, but retained [93]. Some studies in human reported preserved E-cadh expression in high aggressive breast cancer [110], such as the inflammatory breast cancer, in which E-cadh is not only retained but overexpressed [90, 95, 111] and distributed circumferentially 360° around the cancer cell membrane [93]. E-cadh accumulation and, subsequently, overexpression is responsible for the formation of lymphovascular emboli, conferring resistance to apoptosis and a survival advantage. It can be argued that, in the setting of the lymphovascular tumoral embolus, E-cadh is functioning not as a suppressor gene but rather as an oncogene [90]. More recently, Chu et al. [112] showed that E-cadh expression plays an important role in regulating tumourigenicity and hypoxia responses in an inflammatory breast cancer model: the loss of E-cadh and/or overexpression of its repressors, such as ZEB1, downregulated the expression of hypoxia-inducible 1α transcription factor (HIF-1α), leading to a reduction of the extracellular acidification of inflammatory breast cancer, tumour growth and metastasis formation [113].

Although multiple studies reported that metastases were more common in women with breast cancer with absent or low E-cadh expression [91, 114], recent studies have called into question whether cancer cells require the loss of E-cadh to invade and metastasize [89]. Besides, results on the expression of E-cadh in both primary tumour and lymph node metastasis are contradictory. Some studies reported lymph node metastasis with reduced or loss of E-cadh expression [115], whereas others reported overexpression [83, 116] or a similar expression to that observed in the primary tumour [83, 114]. E-cadh overexpression in canine mammary lymph node metastases was also reported by some studies [66, 70]. In the female cat, differences in E-cadh pattern of expression between primary tumors and regional metastases were described [69, 109], suggesting that during mammary carcinogenic progression, there is a dynamic and reversible modulation of the E-cadh complex [69]. E-cadh overexpression in metastases might be associated to their stabilization in the new environment, in order to adhere and to re-establish tissue architecture [116]. Although the mechanism behind this re-expression remains unclear [70], it has been suggested that E-cadh expression is not usually inactivated but its expression is dynamically modulated during the metastatic cascade. This plasticity is probably related to the flexibility of adhesion complexes, which might be temporarily downregulated or not expressed in primary tumours allowing cell detachment and invasion, and lately recovered in the metastatic site, thus favoring the survival and growth of metastatic cells [66, 116]. So, the loss of E-cadh expression might be a transient phenomenon that allows malignant cells to invade vascular channels and tissues; once in circulation, cancer cells re-express E-cadh, facilitating intercellular adhesion and enabling the formation of cohesive tumour emboli [95].

As described previously, cancer invasion and metastasis are, in fact, highly versatile processes, regulated at multiple levels. Recent studies indicate that cancer cells utilize two major migratory strategies: preserving intercellular cohesion as a collective, or as single-cell invasion into the surrounding stroma. The process of cancer cell individualization and acquisition of an invasive migratory phenotype commonly occurs within

the framework of EMT. During EMT, epithelial cells undergo major transcriptional and morphological transformations, resulting in the loss of their intercellular adhesions, and the acquisition of mesenchymal-like properties [117]. As EMT progresses, the transformed cells lose the junctional connections with their neighbours, disengage from the epithelial layer in which they originated, and express mesenchymal markers such as N-cadh, vimentin, and a multitude of specific transcription factors (e.g., Snail, Slug, Twist) [117, 118]. The acquired mesenchymal phenotype is manifested in enhanced migratory activity, extracellular matrix production, invasiveness, and elevated resistance to apoptosis [118, 119]. These changes enable the cells to enter into small vessels and disseminate to distant organs, where they form metastasis [117].

There is controversy about the corollary of the type of E-cadh inactivation (gene mutation or promoter hypermethylation/repression) and the aggressiveness of tumour cells [87]. The infiltrating lobular carcinoma of human breast is classically characterized by a loss of E-cadh immunostaining and intercellular cohesion that results from truncating mutations or epigenetic bi-allelic silencing of E-cadh gene [89, 120-122].

In some breast carcinomas, invasion and metastasis is promoted when E-cadh expression is lost by the promoter methylation or repression by Snail/Slug and other EMT mediators [87, 90]. EMT has been associated with the metastatic cascade in several types of carcinomas, including human breast carcinomas [123, 124]; Lombaerts et al. [87] suggest that E-cadh promoter methylation, but not mutational inactivation, is part of the EMT programme, resulting in increased invasiveness and tumourigenic capacity in breast cancer. The molecular events of this programme can be inferred from the differentially expressed genes and include genes from the TGFβ pathway, transcription factors involved in E-cadh regulation (i.e., ZFHX1B, SNAI2, but not SNAI1, TWIST), annexins, AP1/2 transcription factors and members of the actin and intermediate filament cytoskeleton organization. Altered expression of these transcription factors seems to be also associated with an altered overexpression of transcriptional repressors of E-cadh in tumour cells; thus, considering that metastasis is facilitated by

EMT, the disturbance of this process might prevent breast cancer dissemination [87].

Recently, Raposo-Ferreira et al. [125] provided evidences that EMT plays an important role in the metastatic process of canine mammary carcinomas, describing a significantly high co-expression of E-Cadh$^+$/ vimentin$^+$ in primary mammary carcinomas, especially in high grade carcinomas, when compared to their paired metastases. This distinct expression pattern suggests that EMT is also a dynamic reversible process in canine mammary tumours [125]. In fact, accumulating evidence supports a phenotypic plasticity of metastatic cells that allows a reverse process at a secondary site, known as mesenchymal-epithelial transition (MET), that promots a transition back into an epithelial phenotype which will allow secondary tumour growth [27].

A few studies investigated the expression of EMT-inducing transcription factors, such as Snail, Slug or Zeb, and their correlation with E-cadh in canine or feline mammary neoplasia. With regard to Snail, Im et al. [126] found no association between Snail and E-cadh expression, although Snail expression in canine mammary tumours was significantly correlated with aggressive clinicopathological features such as histological grade and lymphatic invasion. In contrast, Gamba et al. [127] reported a direct association between E-cadh downregulation and Snail up-regulation in canine invasive micropapillary mammary carcinoma; however, no significant correlation was found between E-cadh and Zeb2 in this histological type [128]. Pang et al. [129] demonstrated that EMT induction by TGFβ can enrich cancer cells with stem cell properties.

Classic EMT is usually defined by morphological changes combined with the loss of E-cadh. However, *in vitro* studies using human breast cancer cell lines showed that EMT might be possible without E-cadh loss [130]. Timmermans-Sprang and collaborators [131] described that P-cadh mutations are associated with an EMT phenotype in canine mammary cell lines with E-cadh expression. In fact, in human breast cancer, the presence of P-cadh in an E-cadh positive background can promote invasion [132].

Regarding feline mammary tumours, Buendia et al. [133] found a negative association between E-cadh and N-cadh expression and reported

an association between N-cadh and the tumour histological grade and regional metastasis; these authors suggested that N-cadh expression could be considered a sign of malignancy. Another study showed a co-expression of E-cadh and P-cadh in both primary and metastastic lesions and reported a large number of P-cadh positive feline mammary tumours suggesting that cadherins switching to P-cadh could play an important role in feline mammary tumourigenesis [134]. This hypothesis deserve additional investigation.

CONCLUSION

E-cadh is a classic cadherin that plays an important role in epithelial cell-to-cell adhesion, with recognized functions in morphogenetic processes and in the maintenance of normal tissue architecture. Alterations of E-cadh expression/function have been reported in physiological processes such as throughout the oestrous cycle or during the embryo-maternal interaction at embryo implantation. In contrast, E-cadh loss or reduced expression/function has also been associated with disease, namely with tumourigenesis and malignant progression of carcinomas. Breast cancer is one of the most studied cancer types regarding E-cadh relevance in the tumourigenesis, tumour progression and prognosis. Although the significant number of *in vitro* and *in vivo* investigations in this subject, several questions are still controversial. There is no consensus as to consider E-cadh as a prognostic marker, and its importance during tumour progression differs across the available literature. Nevertheless, E-cadh loss has long been described as a hallmark of EMT, a dynamic process that is especially relevant for breast cancer neoplastic invasion, progression and metastasis formation. In the veterinary setting, although fewer studies are available, especially for the endometrial cancer, results seem similar to the ones described for humans. Future studies are welcome, aiming the factual relevance of E-cadh in the metastatic cascade, focusing in the complex tumour-microenvironment interactions, both at the primary tumour and at the metastatic niche.

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