

## REVIEW

# Expression of cell adhesion molecules in oesophageal carcinoma and its prognostic value

K S Nair, R Naidoo, R Chetty

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Oesophageal carcinoma remains a disease of poor prognosis. Surgical cure rates are compromised by the fact that most patients are diagnosed at a late stage of disease because of the delayed onset of symptoms, by which time metastases and organ infiltration may have already occurred. Thus, invasion and metastases play a key role in influencing patient survival, and the search for novel treatments may therefore hinge on gaining insight into the mechanisms controlling these processes. It has been established that the initial step in the metastatic cascade is the detachment of tumour cells from the primary tumour via dysregulation of normal cell–cell and cell–matrix interactions. Distinct proteins known as cell adhesion molecules (CAMs) mediate these interactions. In recent years, a plethora of information has contributed to the in depth understanding of these molecules. This review provides a brief description of five families of CAMs (cadherins, integrins, CD44, immunoglobulin superfamily, and selectins) and highlights their altered expression in relation both to prognosis and tumour behaviour in squamous cell carcinoma and adenocarcinoma of the oesophagus.

with lymph node metastases or organ infiltration.<sup>2</sup> This implies that the ability of tumour cells to survive and grow at metastatic sites considerably increases the level of morbidity. Therefore, elucidating the mechanisms controlling invasion and metastasis in oesophageal carcinoma may greatly assist in identifying those patients at higher risk of metastases and mortality. This in turn might help in the design of new strategies for diagnosis and treatment of the disease, thereby allowing for an improvement in survival rate.

Recently, there has been a growing interest in investigating various molecular markers in oesophageal carcinoma as potential prognostic, diagnostic, and perhaps therapeutic tools. “Molecular histology”, a term introduced by Edelman and Crossin in 1991,<sup>3</sup> characterises the morphological features of a tissue in terms of the molecules present and the functional interactions between them. Although cancer invasion and metastasis form an intricate process, the initial step is the detachment of neoplastic cells from the primary tumour, followed by entry and exit of the lymphatic or vascular systems and, finally, growth at distant tissue sites.<sup>4</sup> This metastatic cascade of events stems from the dysregulation of normal cell–cell adhesion and cell–matrix interactions. Such interactions are mediated by at least five families of cell adhesion molecules (CAMs), namely: integrins, immunoglobulins (IgCAMs), CD44, selectins, and cadherins (fig 1). Apart from regulating cell–cell and cell–matrix interactions, CAMs also influence cell motility, migration, signalling, and differentiation, in addition to apoptosis and gene transcription. In our present article, we provide an overview of selected CAMs and highlight recent data on the role of their expression in the regulation of invasion and metastasis in oesophageal squamous cell carcinoma (OSCC) and adenocarcinoma (OAC), and its prognostic relevance.

Oesophageal carcinoma is known for its extremely aggressive clinical behaviour and, despite improvement in surgical intervention and preoperative management, the overall prognosis for patients is poor.<sup>1</sup> The assessment of prognosis through clinicopathological characterisation remains inadequate using standard grading and staging systems because of the considerable variability and heterogeneity within different tumours and stages.

“Elucidating the mechanisms controlling invasion and metastasis in oesophageal carcinoma may greatly assist in identifying those patients at higher risk of metastases and mortality”

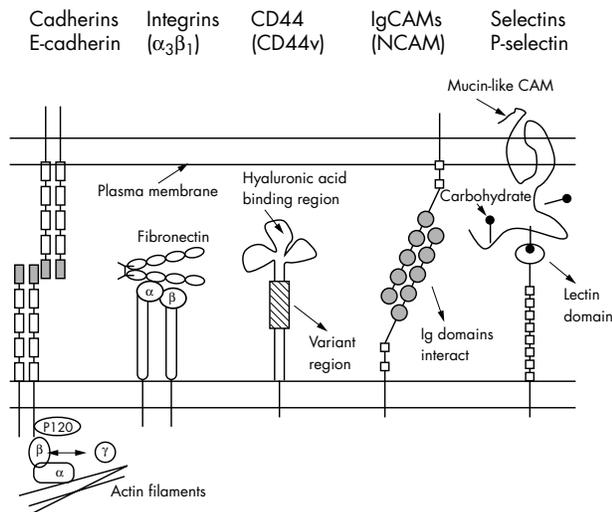
Currently, staging of the disease is the most crucial parameter for predicting survival and recurrence.<sup>2</sup> Patients with minimal tumour invasion of the oesophageal wall and without metastases at the time of resection have a significantly better chance of survival than those

See end of article for authors' affiliations

Correspondence to: Professor R Chetty, University Health Network, Princess Margaret Hospital, 610 University Avenue, Fourth Floor, Suite 302, Room 312, Toronto, ON M5G 2M9, Canada; runjan.chetty@uhn.on.ca

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**Abbreviations:** APC, adenomatous polyposis coli; CAM, cell adhesion molecule; CD44s, standard isoform of CD44; CD44v, variant isoform of CD44; CEA, carcinoembryonic antigen; DCC, deleted in colon cancer; ECM, extracellular matrix; E/N/P-cadherin, epithelial/neuronal/placental cadherin; ICAM, intercellular cell adhesion molecule; Ig, immunoglobulin; MadCAM, mucosal addressin cell adhesion molecule; NCAM, neural cell adhesion molecule; OAC, oesophageal adenocarcinoma; OSCC, oesophageal squamous cell carcinoma; PECAM, platelet–endothelial cell adhesion molecule; VCAM, vascular cell adhesion molecule



**Figure 1** Diagrammatic representation of five families of cell adhesion molecules (CAMs): cadherins, integrins, CD44, immunoglobulin (Ig) CAMs, and selectins. Cadherin dimers engage in calcium dependent homotypic binding to dimers from an adjacent cell. The cytoplasmic domain of E-cadherin is complexed with  $\beta/\gamma$  catenin, which in turn is linked with the actin cytoskeleton via  $\alpha$  catenin. Integrins are heterodimers consisting of  $\alpha$  and  $\beta$  subunits that bind to extracellular molecules. The CD44 standard isoform binds to hyaluronic acid; however, the inclusion of combinations of variant (v) exons alters its binding affinity for this molecule. IgCAMs are characterised by their extracellular Ig-like domains. Selectins possess an N-terminal lectin domain that binds fucosylated and sialylated carbohydrates. NCAM, neural cell adhesion molecule.

## CELL ADHESION MOLECULES

### Cadherins

Cadherins are a family of integral membrane glycoproteins, which are the prime mediators of calcium dependent cell–cell adhesion in normal cells.<sup>3</sup> When cadherins are expressed, the inactivation of other cell–cell adhesion molecules has little or no effect.<sup>6</sup> Binding between cadherins occurs homotypically, with identical molecules binding on adjacent cell surfaces in a “zipper” conformation.<sup>7</sup> The cadherin family is divided into three subfamilies: classic cadherins, which are involved in adherens junctions; and desmocollins and desmogleins, which are both components of desmosomes. Adherens junctions and desmosomes are specialised adhesive sites along the plasma membranes of cells, which bind actin microfilaments and intermediate filaments, respectively.

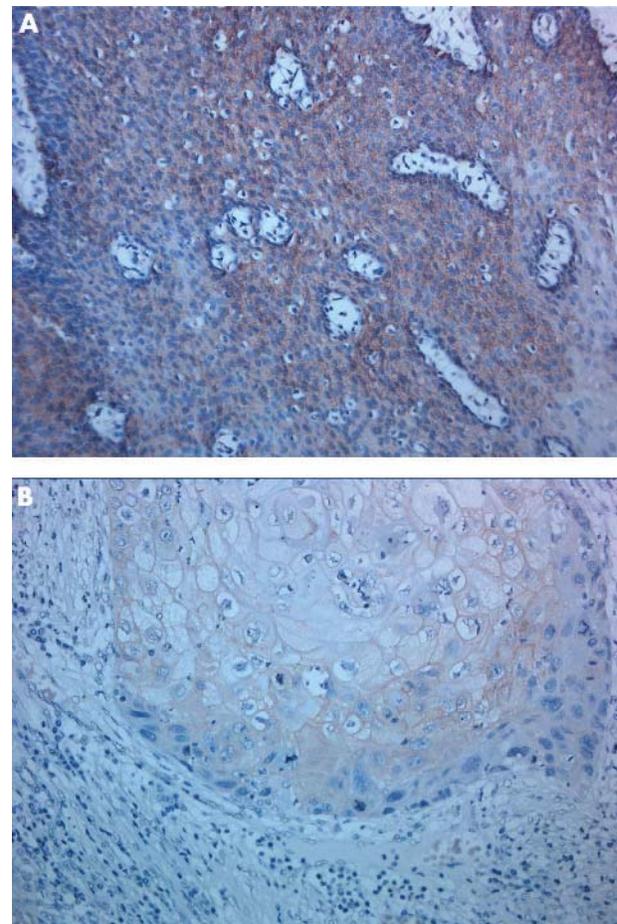
More than 10 subclasses of cadherins have been identified and include E-cadherin/uvomorulin, N-cadherin/A-CAM, P-cadherin, R-cadherin, etc.<sup>8–11</sup> Although all the subclasses share similar features, such as molecular weight and calcium dependence, each subclass is distinguished by a unique tissue distribution pattern. E (epithelial)-cadherin is found in all normal epithelia, P (placental)-cadherin is expressed by trophoblasts and to a minor degree in stratified squamous epithelia, and N (neuronal)-cadherin is expressed mainly in neural tissue.

“Reduced E-cadherin expression was shown to promote epithelial cell invasiveness, dedifferentiation, and metastasis in various human carcinomas”

The most thoroughly researched proteins within the cadherin family are N-cadherin and E-cadherin, which belong to the subfamily of classic cadherins. Classic cadherins play an important role in tissue formation and maintenance during embryonic development, and in the

induction and maintenance of normal architecture and function in adult tissues. Immunological studies capable of disrupting cell–cell adhesion were used to identify E-cadherin in human epithelial tissue. Since then, E-cadherin has been a primary focus for investigation in this group of CAMs. E-cadherin localises to zonula adherens, which are adherens junctions typically found in epithelial cells. Normal E-cadherin expression plays a key role in the maintenance of epithelial integrity and polarised function.<sup>12–13</sup> Reduced E-cadherin expression was shown to promote epithelial cell invasiveness, dedifferentiation, and metastasis in various human carcinomas, supporting a role for this protein as an “invasion suppressor molecule”.<sup>14–16</sup>

The cytoplasmic domains of E-cadherin and N-cadherin associate with a closely related but distinct group of proteins known as catenins ( $\alpha$  catenin,  $\beta$  catenin,  $\gamma$  catenin (plakoglobin), and p120). The catenins tether E-cadherin to the actin cytoskeleton. Deletions and mutations of the catenin binding region of E-cadherin have clearly shown that this region is essential for its adhesive function.<sup>17–18</sup> Genetic alterations of the genes encoding the catenins (particularly  $\beta$  catenin) that produce truncated proteins missing particular binding domains or that alter expression of these proteins lead to reduced cell–cell adhesivity, stemming from adherens junction disassembly (fig 2).<sup>19–21</sup> Both  $\beta$  catenin and  $\gamma$  catenin



**Figure 2** Expression of  $\alpha$  catenin protein. (A) Normal squamous epithelial tissue stained for  $\alpha$  catenin, demonstrating strong staining along the cell–cell boundaries (original magnification,  $\times 200$ ). (B) Immunohistochemical staining of  $\alpha$  catenin in oesophageal squamous cell carcinoma showing reduced expression of  $\alpha$  catenin in the infiltrating cancer cells relative to the normal tissue (original magnification,  $\times 200$ ).

form mutually exclusive complexes with the cytoplasmic tail of E-cadherin.<sup>22–23</sup>

Next,  $\alpha$  catenin links bound  $\beta$  catenin and  $\gamma$  catenin to the actin filaments of the cell cytoskeleton via interactions with various actin binding proteins or by binding actin itself.<sup>24–26</sup> The p120 protein, however, binds at a unique site directly to E-cadherin that is bound to either  $\beta$  catenin or  $\gamma$  catenin without linking it to the actin cytoskeleton.<sup>24–26</sup> The p120 protein has been reported to act as an inhibitor of cadherin mediated adhesion, although the actual mechanism of inhibition is unclear.<sup>27</sup>

The  $\beta$  catenin molecule interacts with the adenomatous polyposis coli (APC) tumour suppressor protein, glycogen synthase kinase  $\beta$ , and an adaptor protein axin (or a homologue, conductin) in a complex, which is responsible for its degradation.<sup>28</sup> This destruction complex is essential to maintain appropriate concentrations of free cytoplasmic  $\beta$  catenin. APC and E-cadherin have been shown to compete for the same binding region on  $\beta$  catenin.<sup>29</sup> It has been suggested that APC modulates the interactions between cadherins and catenins, thereby affecting the pathway through which cellular adhesion controls cell growth and differentiation. In addition to being involved in cell adhesion,  $\beta$  catenin functions in cell signalling via the Wnt–Wingless pathway.<sup>30–31</sup> When a mitotic signal is received by the Wnt–Wingless pathway, a downstream cascade of events leads to the release of  $\beta$  catenin from APC, resulting in a pool of cytoplasmic  $\beta$  catenin. The free  $\beta$  catenin either links to E-cadherin or participates in the Wnt–Wingless pathway by heterodimerising with transcription factors. These complexes then translocate to the nucleus, where they influence the transcription of genes such as the c-MYC oncogene and the cyclin D1 cell cycle regulator.<sup>32</sup> Mutations in the components that regulate  $\beta$  catenin turnover (for example, APC and axin) and mutations in  $\beta$  catenin itself that compromise its degradation have been found in a variety of human tumours.<sup>33</sup> These mutations cause an increased pool of  $\beta$  catenin, which when not rapidly degraded, accumulates in the nucleus. Because the same domain mediates the interaction of  $\beta$  catenin with E-cadherin and transcription factors, these interactions are mutually exclusive. Thus, the recruitment of  $\beta$  catenin into adherens junctions may be inhibited, resulting in decreased cell–cell adhesion.

Although cadherin binding is not a direct activator of Wnt–Wingless signal transduction, recently it has been shown that classic cadherins function as receptors in GTPase signalling.<sup>34–35</sup> In particular, the small GTPases of the Rho family have been identified as part of a membrane local signalling process that regulates cell actin dynamics, cell motility, and adherens junction assembly in response to cadherin adhesion.<sup>36–37</sup> It is thought that cadherin adhesion may influence the precise sites at the plasma membrane where Rho family signalling occurs.

### Integrins

Integrins are a family of ubiquitously expressed transmembrane glycoproteins that exist as  $\alpha/\beta$  heterodimers found at cell adhesion sites known as focal adhesion contacts.<sup>38</sup> The extracellular domains of integrins function as cell surface receptors for extracellular matrix (ECM) molecules, whereas their intracellular domains connect directly or indirectly via linker proteins to the actin cytoskeleton.<sup>38–39</sup> They are pivotal in controlling cell attachment, cell migration, cell cycle progression, and apoptosis, which they regulate in tandem with other signalling pathways.<sup>40</sup> Furthermore, integrins play a role in organogenesis, tissue remodelling, thrombosis, and leucocyte migration.<sup>38–41–42</sup> Integrins must be stimulated to undergo binding to the ECM. Activation of integrins may occur via local stimuli from soluble mediators (such as

hormones, cytokines, growth factors, etc) or by solid interfaces (ECM or other cells).<sup>43–44</sup> Inactivation of integrin mediated adhesion is also necessary to prevent binding at inappropriate times or locations.

There are 18  $\alpha$  and eight  $\beta$  subunits, which dimerise to yield at least 24 molecular permutations, each with distinct ligand binding and signalling properties. These heterodimers may serve as receptors for multiple ligands, but individual dimers recognise separate parts of the same ligand.<sup>38</sup> Despite the high degree of apparent redundancy, most integrins seem to have specific biological functions, particularly during development, raising the possibility of signalling differences between integrins.<sup>45</sup> The integrins are functionally classified into groups according to their  $\beta$  chain. For example, integrins belonging to the  $\beta_1$  group are involved in the adhesion of cells to ECM structural molecules (such as collagen, laminin, merosin, vitronectin, and fibronectin), whereas the  $\beta_2$  integrins (LFA-1, Mac-1, and p150,95), which are expressed solely on leucocytes, function in leucocyte migration to sites of inflammation by binding to members of the immunoglobulin superfamily.<sup>46–47</sup>  $\beta_3$  Integrins have been identified on platelets and influence platelet activation and thrombosis.  $\beta_3$  Integrins have also been identified on platelets and influence platelet activation and thrombosis, whereas,  $\beta_4$  integrins are a major component of hemidesmosomes, which are stable adhesive structures found in the basal layer of stratified and complex epithelia. The  $\beta_1$ ,  $\beta_3$ ,  $\beta_4$ ,  $\beta_5$ , and  $\beta_6$  integrins have all been identified in breast cancer, where their expression may influence the metastatic process.<sup>48–51</sup>  $\beta_7$  Integrins have been shown to be important for homing of intraepithelial lymphocytes to gut mucosa, and  $\beta_8$  integrin subunits form ECM receptors on chick sensory neurones.<sup>52</sup>

“Integrins are pivotal in controlling cell attachment, cell migration, cell cycle progression, and apoptosis”

It has been reported that integrins function in signalling by two mechanisms “inside out” signalling and “outside in” signalling.<sup>53</sup> Inside out signalling is the process by which a cell alters the adhesive state of its integrin receptors, allowing it to bind to other proteins that can modulate the integrin activity state. Outside in signalling transmits signals from the ECM after integrin ligation, which can influence vital cellular processes, such as gene transcription.<sup>54</sup>

In vitro studies have provided evidence of crosstalk between integrins and cadherins: increased integrin expression is seen in keratinocytes in response to the inhibition of cadherin mediated adhesion.<sup>55–56</sup> It has also been shown that the expression of E-cadherin and  $\alpha_2\beta_1$  integrin, a collagen receptor expressed by epithelial cells, is essential for the induction of colorectal tumour cell differentiation in vitro.<sup>57</sup>

### CD44

The CD44 transmembrane glycoproteins bind to ligands (hyaluronic acid, osteopontin, collagen, and laminin) in the ECM, thus mediating cell–matrix adhesion.<sup>58</sup> They have been reported to be involved in lymphocyte homing, T cell activation, and hyaluronic acid binding.<sup>58–60</sup> These proteins allow for the attachment of circulating lymphocytes to vascular endothelium, in addition to binding sedentary epithelial and stromal cells to each other or to the intercellular matrix. The CD44 proteins consist of a large extracellular domain, a membrane spanning region, and a cytoplasmic tail. The cytoplasmic tail of CD44 has recently been localised with linker proteins that connect components of the plasma membrane with the actin cytoskeleton of the cell, suggesting a role for CD44 in cell motility and migration.<sup>61–62</sup>

The human CD44 gene is composed of 21 exons, 10 of which are expressed collectively on all cell types to produce a standard isoform known as CD44s or CD44H. The remaining exons (v1–10) are alternatively spliced and incorporated into the standard protein backbone to create several variant protein isoforms of CD44 (CD44v), which are expressed in various tissues at particular periods during development. The isoforms can be modified further by post-translational glycosylation. The inclusion of different combinations of the v1–10 exons can alter the affinity of CD44 for its principal ligand, hyaluronic acid, allowing for its interaction with other ligands.<sup>58</sup>

Upregulation in the expression of certain CD44v isoforms by cancer cells has been associated with the ability of these cells to metastasise and is also linked with a poor prognosis.<sup>63–65</sup> Soluble forms of CD44 may be detected in the serum of patients with cancer, and in some cases correlate with clinical markers for the disease.<sup>66</sup> The exact process by which tumour cells produce more soluble CD44 is unclear, but it has been suggested that it could result from splicing errors, leading to the production of truncated proteins without a transmembrane region, which would therefore be secreted by the cells.<sup>63</sup>

### Immunoglobulin superfamily

IgCAMs are a large group of cell surface glycoproteins characterised by their extracellular Ig-like domains.<sup>42–67</sup> Included within the Ig superfamily are molecules that function in cellular immunity (major histocompatibility molecules, CD4, CD8, and the T cell receptor), molecules involved in signal transduction (colony stimulating factor 1 and platelet derived growth factor receptor), in addition to the molecules believed to be vital in cell adhesion: neural (NCAM), vascular (VCAM), intercellular (ICAM), platelet–endothelial (PECAM), mucosal addressin (MadCAM), and carcinoembryonic antigen (CEA). Because all these molecules are important in the immune response of T cells, a loss or reduction in their expression might hinder contact inhibition and act as one pathway by which neoplastic cells are able to avoid recognition and elimination. The IgCAMs are capable of both homotypic binding (NCAM binding to NCAM) and heterotypic adhesion (VCAM-1 on endothelial cells binds to VLA-4 ( $\alpha_4\beta_1$ ) integrin on lymphoid cells).<sup>68</sup> Because of the broad diversity that exists in the Ig superfamily, the members of this family are explained individually according to their function in various tissue cell types.

### Neural cell adhesion molecules

NCAMs are expressed in a wide variety of cells types, mainly of neural and mesenchymal origin, where they play a crucial role in the preservation of the integrity of the nervous system.<sup>69</sup> It has been reported that downregulation of NCAM expression is associated with a reduction in contact inhibition in a mouse fibroblast cell line,<sup>70</sup> but this has not been demonstrated in vivo in a variety of human cancers. However, Seki and co-workers<sup>71</sup> reported a significant correlation between NCAM expression and perineural invasion in bile duct cancer, where carcinoma cells invade the perineural space by recognising NCAM expressed on neural cells. Furthermore, NCAM has been visualised histochemically on many neural and neuroendocrine tumours.<sup>72</sup> The form of NCAM present on these tumours is the embryonic form containing high quantities of polysialic acid. This isoform of NCAM is less adhesive than the adult form and may be relevant to the metastatic potential of the cells.

“Neural cell adhesion molecule has been visualised histochemically on many neural and neuroendocrine tumours”

The deleted in colon cancer (DCC) gene, which has been identified as a tumour suppressor gene for colorectal cancer and various other tumours including those of the stomach, pancreas, oesophagus, and bladder, produces a protein that is structurally similar to NCAM.<sup>73</sup> In vertebrate adult tissues, the highest concentrations of DCC protein are found in the brain and neural tissues, where it functions in the guidance of developing axons.<sup>74</sup> The DCC gene is also expressed in most epithelial tissues (such as the gut, skin, lung, etc), particularly in proliferating cells as the epithelia mature, where the protein may participate in cell–cell or cell–substratum interactions. Decreased or absent expression of the DCC gene has been reported to result in liver metastases in colorectal carcinoma<sup>75</sup> and disease dissemination in neuroblastomas.<sup>76</sup> Allelic loss at the DCC gene locus has been implicated in lymph node metastasis in OSSC<sup>77</sup>; however, DCC expression in this tumour type has not been reported.

### Vascular cell adhesion molecules

VCAM-1 is involved in the adhesion of lymphocytes, monocytes, and eosinophils to the endothelium through binding with its ligands,  $\alpha_4\beta_1$  and  $\alpha_4\beta_7$  integrins.<sup>68–78</sup> The expression of VCAM-1 on endothelial cells is induced by cytokine activation. Tissue necrosis factor  $\alpha$ , lipopolysaccharide, and interleukin 1 are all responsible for the upregulation of VCAM-1 and ICAM-1 expression. Decreased VCAM-1 expression has been shown to be associated with metastatic behaviour in melanoma cells.<sup>47</sup> Raised concentrations of the soluble form of VCAM-1 have been reported in patients with cancer.<sup>79</sup>

### Intercellular cell adhesion molecules

Structurally, there are three types of ICAMs on leucocytes: ICAM-1, ICAM-2, and ICAM-3.<sup>67</sup> The ICAMs bind to leucocyte integrins, LFA-1 and Mac-1.<sup>80</sup> The expression of ICAM-1 on vascular epithelium is a typical feature of the inflammatory immune response, mediating the interactions of leucocytes and endothelial cells by binding to integrins. ICAM-1 is expressed in various types of squamous cell carcinomas and melanomas. In particular, ICAM-1 expression in malignant melanoma cells has been linked to increased risk of metastasis.<sup>81</sup>

### Platelet–endothelial cell adhesion molecules

PECAMs function in leucocyte adhesion, homotypic binding, and heterotypic binding with  $\alpha_5\beta_3$  integrin.<sup>82</sup> PECAM-1 is expressed in large amounts on vascular endothelial cells at intercellular junctions, on platelets, and on some leucocytes.<sup>83–84</sup> Because PECAM-1 is localised at the intercellular junctions of endothelial cells, it is thought to play a major role in the maintenance of endothelial integrity and the control of leucocyte migration across the endothelial lining. In a study conducted by Tang *et al*,<sup>85</sup> it was shown that tumour cell PECAM-1 mediates tumour cell adhesion to the endothelium.

### Mucosal addressin cell adhesion molecules

MadCAM-1 contains Ig domains homologous to ICAM-1 and VCAM-1, in addition to a unique, highly glycosylated region. It is able to bind both  $\alpha_4\beta_7$  integrin and L-selectin.<sup>78–86</sup> The molecule participates in lymphocyte homing to venules of lymph nodes and mucosal lymphoid tissue.

### Carcinoembryogenic antigen

CEA was originally identified in high concentrations in the serum of patients with colon carcinoma,<sup>87</sup> and has subsequently proved to be a clinically useful marker of early or recurrent colorectal and lung cancer. CEA is attached to the cell membrane by a glycoprotein anchor, and is probably

released in a soluble form by enzyme action.<sup>88</sup> These molecules function in both homotypic and heterotypic binding.<sup>88-90</sup> However, it is not known whether dysregulation of CEA is causally involved in tumour cell detachment from primary sites and their spread to distant tissues.

### Selectins

The selectins are a group of cell surface lectins that mediate the adhesion between leucocytes, platelets, and endothelial cells under blood flow. Selectin mediated adhesion ensures that leucocytes roll in the direction of flow, which is a prerequisite for recruitment of leucocytes to areas of injury and inflammation. Leucocyte rolling is modulated by selectins recognising and heterotypically binding to mucin-like cell surface glycans that are fucosylated and sialylated—for example, sialylated Lewis<sub>x</sub> antigens.<sup>91</sup> Interestingly, these glycans are expressed on the cell surface of digestive tract cancers and are also present in the soluble form in the circulation of patients bearing these tumours.<sup>92</sup>

At present, the selectin family is small, consisting of three closely related proteins—L-selectin, E-selectin, and P-selectin—expressed by both platelets and vascular endothelium. L-selectin interacts with mucin-like proteins: CD34, glycosylation dependent cell adhesion molecule 1, and MAdCAM-1.<sup>93-94</sup> E-selectin binds to E-selectin ligand 1, although it is also known to interact with P-selectin glycoprotein ligand 1, which is the primary ligand of P-selectin.

The selectins, like members of the IgCAM family (ICAMs and VCAM-1), are expressed in low amounts at the cell surface. When the cells are activated by inflammatory stimuli, P-selectin is released from the storage bodies of endothelial cells and is mobilised to the external plasma membrane.<sup>95</sup> E-selectin is synthesised de novo and transported to the endothelial cell surface upon exposure to a range of inflammatory mediators. L-selectin is expressed by leucocytes, but is also shed from the surface of the cell into the circulation. This increased expression of selectins results in the slowing and rolling of leucocytes along the endothelial cell wall. Signals are then triggered that activate and upregulate leucocyte integrins, which causes the tight adhesion of neutrophils through  $\alpha_4\beta_1$  and  $\alpha_4\beta_7$  integrins binding to endothelial VCAM-1 and MAdCAM-1. Transmigration through the endothelium to sites of injury follows.

E-selectin was shown to regulate the attachment of a colon carcinoma cell line to endothelial cells in vitro,<sup>96</sup> whereas P-selectin has been shown to bind to various types of carcinoma cells.<sup>97</sup> L-selectin has been identified as a possible predictor of the haematogenous dissemination of murine lymphomas.<sup>98</sup> These findings suggest that site specific metastasis may be mediated partly by the ability of blood borne tumour cells to identify and bind to distant endothelial CAMs. These observations also imply that tumour cells during dissemination may use the same CAMs used by normal cells for traffic and localisation in various tissues.

## CAM EXPRESSION IN OESOPHAGEAL CARCINOMA

### Cadherins

#### Oesophageal squamous cell carcinoma

E-cadherin expression has been investigated intensively in oesophageal carcinomas. In a study conducted by Zhou *et al*,<sup>99</sup> E-cadherin and  $\beta$  catenin expression was correlated with clinicopathological variables in OSCC. E-cadherin expression but not  $\beta$  catenin expression determined prognosis. However, another investigation conducted on E-cadherin,  $\alpha$ ,  $\beta$ , and  $\gamma$  catenin expression in OSCC reported that, although the reduction in expression of all these molecules correlated closely with tumour grade,  $\alpha$  catenin was a better predictor of prognosis.<sup>100</sup> Furthermore, Kadowaki and co-workers<sup>101</sup> have also suggested that  $\alpha$  catenin expression is more closely

correlated with an invasive phenotype and lymph node metastasis than E-cadherin expression in OSCC. These results imply that the expression of  $\alpha$  catenin may be downregulated earlier than E-cadherin during malignant transformation. Nagafuchi *et al* showed that E-cadherin expression regulated the degradation of  $\alpha$  catenin post-transcriptionally in vitro, so that the loss of  $\alpha$  catenin expression might act as a more sensitive indicator of the loss of E-cadherin function.<sup>102</sup>

Reduction in the expression of E-cadherin in patients with OSCC was shown to be strongly associated with postoperative blood borne recurrence, resulting in a poorer prognosis than in those patients with tumours showing normal expression before surgery.<sup>103</sup> This finding suggested that in patients with reduced E-cadherin immunoreactivity, the metastatic potential of the oesophageal cancer cells may be increased. Therefore, the evaluation of E-cadherin immunoreactivity may be useful in predicting haematogenous spread and hence recurrence, thus serving as an aid for planning adjuvant treatment after surgery in patients with OSCC. It has also been reported that E-cadherin might be an independent predictor of micrometastasis in lymph nodes that are classified as N0 by routine histopathological analysis.<sup>99-104</sup>

### Oesophageal adenocarcinoma

The analysis of E-cadherin,  $\alpha$  catenin, and  $\beta$  catenin expression in OAC revealed that reduced expression of all three proteins correlated with decreased patient survival.<sup>105</sup> In addition, the expression of E-cadherin and  $\beta$  catenin is significantly correlated with poor prognosis, and therefore may be used to identify patients with a higher risk of disease recurrence. In some cases, the staining pattern of the E-cadherin–catenin complex does not always show an absence or reduction in expression, but may indicate a redistribution from the cell membrane to the cytoplasm.<sup>106</sup> The process responsible for this redistribution is still unclear; however, a change in phosphorylation status has been cited as a probable cause. Tyrosine phosphorylation of  $\beta$  catenin in particular is known to prevent the association of E-cadherin with  $\alpha$  catenin, despite normal E-cadherin expression.<sup>107</sup> This suggests that even though the proteins may be expressed, this does not necessarily imply that they are functioning normally.

‘The analysis of E-cadherin,  $\alpha$  catenin, and  $\beta$  catenin expression in oesophageal adenocarcinoma revealed that reduced expression of all three proteins correlated with decreased patient survival’

Some investigators have highlighted certain components of the cadherin–catenin complex as having more prognostic value than others. Considering that all the components of the complex are dependent on each other to maintain normal cell–cell adhesion, it is useful to investigate the expression of both E-cadherin and the catenins. It is evident that more studies have to be performed on the cadherin–catenin complex before it can be used for routine analysis, particularly with regard to the role of these molecules in cell signalling pathways.

### Integrins

#### Oesophageal squamous cell carcinoma

In another study, Miller and Veale<sup>108</sup> found that OSCCs expressed the  $\alpha_v$  integrin subunit strongly, whereas normal oesophageal tissue did not express this protein. Therefore, the  $\alpha_v$  integrin subunit may be regarded as a significant indicator of the malignant phenotype. These investigators also reported the downregulation of the  $\alpha_2$  and  $\beta_1$  integrin subunits, which implies that cell adhesion may be weakened, whereas migration is promoted, in OSCC cells as a result of de novo

$\alpha$ v expression. Takayama and colleagues<sup>109</sup> also found that reduced expression of  $\beta_1$  integrin was positively correlated with lymph node metastasis in OSCC. cDNA microarray analysis of OSCC cell lines revealed upregulation of  $\alpha_3$ ,  $\alpha_6$ , and  $\beta_4$  integrins in the tumour cells.<sup>110</sup> Sato *et al*,<sup>111</sup> using two OSCC cell lines TE-1 and T.Tn, identified integrin adhesion as an important step in the blood borne metastasis of oesophageal cancer. They postulated that cancer cells receive stimulation from cytokines produced by endothelial cells after initial selectin mediated adhesion of cancer cells. This increases the expression of cell adhesion molecules, such as the  $\beta_1$  integrin family, leading to an increase in the adhesive activity of cancer cells along vessel walls. This sequence of events may culminate in the enhanced transmigration of cancer cells to extravascular tissues.

### Oesophageal adenocarcinoma

Gene expression associated with OAC was investigated using a cDNA microarray containing 1176 human cancer genes.<sup>112</sup> The expression of  $\alpha_3$  and  $\alpha_6$  integrin was shown to be upregulated in the tumour tissue; however, these data were not correlated with the clinicopathological features. The immunohistochemical analysis of  $\beta_1$  integrins in Barrett's oesophagus revealed no correlation between the expression of the  $\beta_1$  integrins and histomorphological characteristics or survival, indicating that these molecules were of no prognostic value for the disease.<sup>113</sup>

Overexpression of mRNA for  $\alpha_6$  integrin, which is a major receptor for laminin and is known to be involved in tumour cell invasion, was found in 87% of OAC cases investigated.<sup>114</sup> This suggests that  $\alpha_6$  integrin mRNA is overexpressed in human OAC and may influence oesophageal tumour invasion.

However, immunohistological analysis of integrin expression in clinical specimens should be interpreted with care because integrin function cannot always be inferred from its expression alone. The cell on which the integrin is expressed can alter its ligand binding properties without changing its expression levels.<sup>115</sup> Immunohistological analysis is further compromised because changes in the expression of a single integrin subunit have to be judged against the background expression of all other integrins, in addition to the activation state of growth factor and cytokine signalling pathways that regulate integrin function.

### CD44

#### Oesophageal squamous cell carcinoma

The upregulation of CD44v isoforms has been noted as a marker for tumour metastasis and prognosis in several carcinomas.<sup>63 65 116</sup> Downregulation of CD44v has also been noted in squamous cell carcinomas of the head and neck and neuroblastomas.<sup>117</sup> In an immunohistochemical study conducted on 233 patients with OSCC to investigate whether CD44v could be of prognostic value, CD44v2 was found to be downregulated in the cancer cells.<sup>118</sup> Although CD44v2 expression did not correlate with clinicopathological factors, patients with CD44v2 positive tumours survived significantly longer than those with CD44v2 negative tumours. The authors suggested that downregulation of CD44v2 expression is associated with a poor prognosis in patients with OSCC and could be used as a clinical marker to determine the need for adjuvant treatment.

#### Oesophageal adenocarcinoma

The immunoreactivity of CD44s and variants (v4/5, v6) was analysed in Barrett's mucosa and Barrett's adenocarcinoma (Barrett's oesophagus).<sup>119</sup> The pattern of expression shown indicated that CD44s and CD44v6 might be involved in the carcinogenesis of Barrett's mucosa. It was also noted that CD44v6 expression in adenocarcinoma was correlated with

depth of invasion, neoplastic vascular invasion, and neoplastic perineural invasion, but did not influence outcome. In contrast, Bottger *et al* studied the expression of CD44s and CD44v4 in OAC and found that increased CD44v4 was of prognostic value in that its expression correlated with decreased patient survival.<sup>120</sup>

The most promising area for the use of CD44 in clinical diagnosis is in the analysis of body fluids. High levels of accuracy have been achieved in detecting bladder cancer by analysing exfoliated cells in non-invasively obtained urine samples.<sup>121</sup> Analysing gastric or oesophageal luminal contents for CD44v would be highly beneficial in screening patients with both symptomatic and asymptomatic disease; however, this area of research has not been investigated in oesophageal cancer.

### Immunoglobulin superfamily

#### Oesophageal squamous cell carcinoma

CEA has been the most investigated molecule in this group of CAMs, particularly in OSCC, because it has proved useful as a routine marker for another gastrointestinal tract tumour, colorectal cancer.<sup>122</sup> Previous studies have stated that only the more mature cells of stratified squamous epithelia express CEA on their membranes and their immunohistochemical visualisation can therefore be used as a differentiation marker in these epithelia.<sup>123-125</sup> This theory is supported by another investigation that showed a clear correlation between the degree of tumour differentiation and CEA expression for carcinomas of the oesophagus, stomach, and colon.<sup>126</sup> Stromal CEA expression has also been reported to play a role in lymphatic invasion of OSCC.<sup>127</sup>

"The correlation between lymph node metastases and deficient expression of HLA class I antigens indicates that tumour cell spread may be facilitated by a reduced ability of the immune system to recognise these cells"

In a study examining postoperative serum CEA mRNA values in OSCC, it was noted that CEA mRNA positivity was detected most frequently after tumour resection and was associated with nodal status and disease stage.<sup>128</sup> The incidence of total recurrence and blood borne recurrence was significantly greater in patients positive for CEA mRNA than in negative patients. The authors recommended effective adjuvant treatment for patients with OSCC who expressed CEA mRNA after surgery. Rockett *et al* investigated the immunoexpression of class I (HLA-ABC) and class II (HLA-DR) major histocompatibility antigens and ICAM-1 in oesophageal carcinomas.<sup>129</sup> Their study revealed that HLA-DR and ICAM were absent from a high proportion of OSCCs; however, these data did not correlate with tissue differentiation or staging. The biological relevance of HLA-DR and ICAM absence is not known, but it could be related to the suppression of infiltrating lymphocytes. Moreover, Hosch and colleagues<sup>130</sup> reported that the expression of HLA class I and ICAM-1 in oesophageal carcinoma, particularly OSCC, was a significant predictor of reduced risk of developing tumour recurrence, whereas expression of ICAM-1 on HLA class I negative tumours was associated with an increased risk of tumour relapse. The correlation between lymph node metastases and deficient expression of HLA class I antigens indicates that tumour cell spread may be facilitated by a reduced ability of the immune system to recognise these cells.

#### Oesophageal adenocarcinoma

The study by Rockett *et al* also showed that HLA-DR was weakly expressed mostly within the cytoplasm in only 20% of the OAC cases examined, although this did not correlate with

### Take home messages

- Numerous studies strongly highlight the value of the immunoeexpression of the cadherin–catenin complex as a predictor of prognosis, invasive phenotype, and haematogenous recurrence in oesophageal carcinoma
- The interpretation of integrin immunoeexpression patterns is difficult because of the activation state of the integrins and the ability of cells to alter their integrin ligand binding without changing expression levels
- CD44v2 downregulation in oesophageal squamous cell carcinoma (OSCC) and the decreased expression of CD44v4 in oesophageal adenocarcinoma (OAC) correlates with reduced patient survival
- Stromal carcinoembryonic antigen (CEA) immunoreactivity has been shown to be involved in lymphatic invasion of OSCC, and postoperative CEA mRNA positivity is associated with tumour recurrence
- In patients with OSCC and distant lymph node metastasis, increased expression of sialyl Le(a) and sialyl Le(x) antigens is associated with haematogenous disease recurrence

tumour differentiation or staging.<sup>129</sup> HLA-DR and ICAM-1 were also aberrantly expressed in cells from which oesophageal carcinoma was thought to arise. The decreased expression of these molecules, which play a central role in stimulating the immune response, may indicate a partial or inhibited immune response against OSCC and OAC tumour cells, perhaps allowing for the progression of this aggressive malignancy.

### Selectins

#### Oesophageal squamous cell carcinoma

The study of the immunoeexpression of selectins in oesophageal cancer is an area of research that is relatively unexplored. However, sialyl Le(a), which is a ligand for E-selectin, was detected in 51% of OSCCs, regardless of the depth of tumour invasion, vascular invasion, or lymph node status.<sup>131</sup> High sialyl Le(a) expression was detected not only in the intramucosal carcinoma component, but also in the invasive carcinoma components, suggesting that sialyl Le(a) expression is associated with early stage cancer progression. In addition, it has been reported that patients with OSCC who have distant lymph node metastases and increased expression of sialyl Le(a) and sialyl Le(x) antigens may be prone to haematogenous recurrence of the disease.<sup>132</sup>

### CONCLUSIONS

Despite the growing body of knowledge relating to the biology of cell adhesion, the data available from numerous publications make it difficult to define one simple scheme in which cell adhesion can influence oesophageal cancer growth and metastasis. As a result, the diagnostic and therapeutic usefulness of adhesion molecules remains largely untapped. The associations between the various adhesion molecules are probably more complicated than the present review implies, consisting of an intricate array of interactions, regulatory processes, and signalling events. More will need to be learnt about the dialogue between cell adhesion molecules and signalling pathways in oesophageal cancer to create a more dynamic approach to clinical diagnosis and prognosis and therapeutic intervention.

### Authors' affiliations

**K S Nair, R Naidoo**, Pfizer Molecular Biology Research Facility, Doris Duke Medical Research Institute, Nelson R Mandela School of Medicine, University of Natal, Congella 4013, Durban, South Africa  
**R Chetty**, Department of Pathology, University Health Network/Toronto Medical Laboratories and University of Toronto, Toronto, ON M5G 2M9, Canada

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