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Review

KAI-1/CD82, The molecule and clinical implication in cancer and cancer metastasis

Fraz Arshad Malik[1,2], Andrew J. Sanders[1] and Wen G. Jiang[1]

[1]Department of Surgery, Cardiff University School of Medicine, Cardiff, UK and

[2]Departments of Biosciences, COMSATs Institute of Information Technology, Islamabad, Pakistan

Summary. CD82, also known as KAI-1, structurally belongs to tetraspanin family while categorised as metastasis suppressor gene on functional grounds. KAI1/CD82 is localized on cell membrane and form interactions with other tetraspanins, integrins and chemokines which are respectively responsible for cell migration, adhesion and signalling. In recent years apart from its significant involvement in the suppression of secondary tumours it has also been observed that KAI1/CD82 plays a vital role in virus binding and its entry inside the cell. Decreased expression of KAI1/CD82 molecule results in aggravating cancer progression. Altered expression levels of KAI1/CD82 molecule in different types of human cancer have been implicated as having prognostic value and linking to the long term survival of the patients. Increased level of KAI1/CD82 also results in the suppression of secondary tumour growth. Increased expression of this molecule results in reduced cell invasion and cell migration due to endocytosis of epidermal growth factor receptors (EGFR). Thus, KAI-1/CD82 is a pivotal molecule in the regulation of cancer cells' behaviour and has important clinical and therapeutic implications in cancer.

Key words: KAI-1, CD82, Cancer, Cancer metastasis, Invasion

Introduction

KAI1/CD82 is a member of tetraspanin family, also termed as tetraspan TM4SF, tetraspanin TM4-C and is present in eukaryotes (Vogt et al., 2002). Most of member proteins of the TMSF4 family are found in almost all tissues (e.g. CD9); but some are cell type specific, for example, CD37 is largely seen in mature B cells. The primary feature in this family of proteins is that they have four transmembrane and two extracellular domains. A small extracellular domain termed as SED/EC1 and large extracellular domain called LED/EC2. Tetraspanin are differentiated from other transmembrane proteins by conserved domains listed under pfam00335.12 (Hemler, 2005) and conserved CCG motif on ED2 with a cysteine residue proximal to that motif on transmembrane 4 (Seigneuret et al., 2001; Stipp et al., 2003). The family is composed of ~34 members out of whom 33 have been identified in human; with many of them expressing on leukocytes (Tarrant et al., 2003). A number of factors have rendered the familiarity to be difficult in evaluation for this family: firstly their molecular association are quite recent in origin (Rubinstein et al., 1996), secondly their minute protrusion of 4-5nm only make their detection and screening very difficult in various biochemical and immunological assays and thirdly they don't retain a very stable of receptor ligand interactions with few exceptions (Hemler, 2005). Collectively, this family protein is known to be involved in the regulation of cell morphology, cell signalling including cell proliferation, fusion and motility (Skubitz et al., 1996; Lagaudriere-Gesbert et al., 1997) and immune system (Toyo-Oka et al., 1999).

KAI1/CD82 was first identified in T-cell activation study (Gaugitsch et al., 1991). Its role on metastasis suppression in prostate cancer was explored later by somatic cell hybridization of highly metastatic and non metastatic rat prostate cancer cells (Ichikawa et al., 1991). Dong mapped the location of KAI1/CD82 on chromosome 11 in 1995 (Dong et al., 1995). KAI1/CD82 is also termed as KAI (Kangai as from Chinese meaning anti cancer), SAR2 leukocyte surface antigen R2 and suppressor of tumorgenicity 6 ST6.

Offprint requests to: Dr. Wen G. Jiang, Department of Surgery, Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, UK. e-mail: jiangw@cf.ac.uk

Gene structure and mutation spectrum of KAI-1/CD82

Dong et al. (1995) identified the location of CD82/KAI1 gene on chromosome 11p with 10 exons and 9 introns spanning around 80kb. CD82/KAI1 gene has no TAATA box in the upstream, region but has putative binding sites for many transcription factors in the upstream region including SP1 and AP2 binding sites. Coding starts from exon 3 of the gene and translation initiation site is 130 base pair downstream from transcription start site. KAI1/CD82 exists in two isoforms, with 267 amino acid residues in isoform1 and 242 residues in isoform2 (Todeschini et al., 2008). Mutational spectrum of KAI1/CD82 in oesophageal squamous cell carcinoma has been analyzed with the aid of PCR -SSCP in 22 patients. According to this research group, dysregulation of KAI1/CD82 protein is not correlated with mutations of the coding region (Miyazaki et al., 2000). In another study on epithelial ovarian carcinoma, only a single mis sense mutation at codon 241, showing conversion of valine to isoleucine in both normal and tumour tissues was observed (Liu et al., 2000). Similarly, no significant association of loss of heterozygosity (LOH) for KAI1/CD82 genomic region with down regulation of protein in lung adenocarcinoma (Tagawa et al., 1999) was found. Hence, it has been observed that the down regulation of KAI1/CD82 protein is mainly due to dysregulation of some proteins rather than the mutation on the gene itself. Minimal promoter size for KAI1/CD82 is 0.5kb with two regions of prime importance; one starting from -197 to +351 at 5'prime site as a positive regulator while region from -735 to -197 having a negative effect on transcription. Enhancer was located further upstream of the basal promoter region in -922 to -846bp (Gao et al., 2003).

Expression profiling and localisation of KAI1/CD82

KAI1/CD82 protein size ranges from 46-70kD due to glycosylation (White et al., 1998) showing 76% identity and 82% similarity between human and mouse. It indicates conserved spot with respect to its role in evolution as well as in development (Nagira et al., 1994). KAI1/CD82 is found in major histocompatibility complex class II compartments of B-lymphoid cells with DR, DM and DO molecules (Hammond et al., 1998). It is present on cellular membrane and along with several other tetraspanin forming a web like shape termed as tetraspanin web or tetraspanin enriched micro domain (TERM). In normal cells, expression of KAI1/CD82 is ubiquitous and its mRNA was observed in almost every body organ (Hemler, 2001). Transmembrane domain of tetraspanin KAI1/CD82 plays a vital role in progression of signalling cascades in cells. Without transmembrane 1 domain TM1, release of tetraspanin KAI1/CD82 molecule from endoplasmic reticulum is not possible (Cannon and Cresswell, 2001). Regulation of KAI1/CD82 is co-ordinated with number of transcriptional binding factors such as direct relationship between p53 protein and KAI1/CD82 upstream binding portion within 850bp. This may suggest that the loss of p53 consensus binding region and p53 function, which is commonly observed in various types of cancer, leads to down regulation of the KAI1/CD82 gene (Mashimo et al., 1998; Briese et al., 2008). However, a contrasting picture was also seen in which no altered expression of KAI1/CD82 with varying p53 levels after inducing various genotoxicity was also observed, narrating that p53 may not be a transcriptional regulator of KAI1(Duriez et al., 2000). On clinical ground, no correlation among KAI1/CD82 and p53 protein was also observed in Farhadieh's study (Farhadieh et al., 2004). One possible explanation of this variance may be the findings that KAI-1 transcription regulation may be the result of combinational role of transcription factors AP1, AP2 and p53 (Marreiros et al., 2003). Similarly antagonistic effect of ß-catenins and reptins on Co-activator Tip 60 also shed light on the regulatory mechanism of KAI1/CD82 protein (Kim et al., 2005). Promoter methylation was also observed for silencing the tetraspanin especially in case of myeloma cell lines, resulting in silencing of the gene in metastasis progression (Drucker et al., 2006). Another protein named as Fe65 interacts with amyloid precursor protein (APP); acting as anchoring molecule for its entry to nucleus where it initiates expression of KAI1/CD82 by binding with TIP60 and promoter of KAI1/CD82 (Telese et al., 2005). Thus, expressional variations observed on KAI1/CD82 is more due to the coordinated altered regulation of various molecules rather than mutation on the coding region.

Biochemical features of KAI1/CD82

Post translational modification of KAI1/CD82

Tetraspanin homo dimerization occurs in golgi complex (Kovalenko et al., 2004) while in endoplasmic reticulum it associates with uroplakins to form heterodimer complex (Berditchevski, 2001). Although the detailed mechanism regarding KAI1/CD82 role in late endosomal and exosomal (baso-lateral targeting of the cells) activities is yet to be established but it has been observed that sequence YXXΦ is required at carboxy-terminal of any tetraspanin to perform this function (Bonifacino and Traub, 2003). Palmitoylation and glycoslyation on KAI1/CD82 is mandatory for its integration with actin cytoskeleton structure and other tetraspanin molecules (Ono and Hakomori, 2004; Zhou et al., 2004). All 5 cysteines residues of KAI1/CD82 (Cys 5, Cys74, Cys 83, Cys 251, Cys 253) proximal to cell membrane are palmitoylated, which on removal results in decrease of KAI1/CD82 dependent cell migration and invasion (Zhou, et al., 2004) as KAI1/CD82 level responsible for the formation of tetraspanin web is drastically reduced on cell membrane. Glycosylation level may vary with respect to various

tissues but has a potent role in cell motility (Ono and Hakomori, 2004).

KAI1/CD82 associated molecules

KAI1/CD82, apart from its association with CD9 and CD81 in tetraspanin web, is also linked with integrins, immunoglobulin like EWI2 and epidermal growth factor receptors (EFGR) (Hemler, 2005). Integrins are actually a family of heterodimeric transmembrane proteins acting as receptor for extracellular matrix proteins. Integrins and EGFR associated KAI1/CD82 regulation has been very well studied by various independent groups in context to cancer suppression and will be discussed separately in this review. Cytoplasmic portion of KAI1/CD82 showed association with SUMOlyation protein kinase C. KAI1/CD82 molecule also associates with protein kinase C through the cytoplasmic tail and this association is indeed helpful for an indirect phosphorylation of integrins attached to the extracellular loop of tetraspanin proteins. KAI1/CD82 actually works as a linker molecule between integrins and PKC and which in return of phosphorylation are involved in cell adhesion and migration (Zhang et al., 2001). EWI2 is a transmembrane protein belonging to newly identified immunoglobulin superfamily IgSF and is involved in cell-cell interaction and adhesion. EW12 also termed as KAI1/CD82 associated surface protein KASP though it also associates with other tetraspanin. Association between EW12 and KAI1/CD82 has a strong inhibitory effect of cell migration (Zhang et al., 2003). It is interesting to note that KAI1/CD82 has a negative regulatory effect on ErbB thus retaining a control on cancer cell proliferation and metastasis suppression such as breast cancer cells (Sweeney et al., 2006).

Signalling cascade

In one study no direct association of KAI1/CD82 with PI 4 kinase was observed (Yauch and Hemler, 2000) this provides an evidential support of some other regulatory mechanism for cell signalling cascades. In a study on L6 Ag (tumour associated antigen) cell motility effects, it has been observed that its binding to tetraspanin enriched microdomain (TERM) increases the motility of the cells, followed by reduction of CD62 (~2.5 fold) and KAI1/CD82 (40%). The most striking feature of this study is the fact that though over expression of KAI1/CD82 showed alteration in FAK signalling pathway;(no effect on Focal Adhesion kinase but its downstream p130 (Crk associated substrate) showed a significant decrease leading to decrease in cellular motility) was observed but no such association has been observed in relation to L6-Ag. This point highlights the involvement of some other proteins either responsible for ubiquitlaytion on L6-Ag on binding to TERM or KAI1/CD82 ecotopic regulation (Lekishvili et al., 2008). In T-cells it was observed that association of tetraspanin web and actin cytoskeleton depend upon nature of membrane organization and KAI1/CD82 molecule (Delaguillaumie et al., 2004). KAI1/CD82 and CD81 apart from interacting to each other also interact with CD4 on T cells. Interaction of CD4 and CD81 depends upon cytoplasmic tail of CD4 while both cytoplasmic and extracellular domain is required for interaction with KAI1/CD82. KAI1/CD82 acts as antagonist to p56 molecules as preventing T-cells from premature activation (Imai and Yoshie, 1993; Imai et al., 1995). In another study conducted in bladder cancer the association of KAI1, FGFR3, PTEN, EGFR and Ras has been compared in normal and invasive tissues to correlate their sub cellular location and disease relevance as well (Rotterud et al., 2007). Two genes producing cell surface proteins BMPR2 and KAI1/CD82 showed marked increase in expression when HUVEC (human umbilical vein endothelial cell) and hepatic Fao cells are cultured together indicating a cumulative role of adhesion and proliferation (Takayama et al., 2007).

Stability and degradation of KAI1/CD82

Small Ubiquintin like modifiers (SUMO) are responsible for the stability of the various proteins. These proteins compete with Ubiquintin for the same Lysine residue and prolong the cellular haematosis, by increasing the shelf life of target protein. Ubquintin are responsible for degradation of target proteins. Indirect repression of KAI1/CD82 expression is done by direct SUMOlyation of reptin and its association with histone deacetylase 1 (HDAC1) resulting in switching off of KAI1/CD82 expression (Kim et al., 2006). DeSUMOylation of this complex by SUMO-specific protease (SENP1) resulted in release of reptin and later on binding of TIP 60 (coactivator) which leads to switching on of KAI1/CD82 expression (Melchior et al., 2003; Kim and Baek, 2006). In fact involvement of SUMOlyation in cancer progression also opens a new horizon for therapeutic interventions, as Ubc9 only conjugating enzyme, responsible for SUMOlyation process can act as a drug targeted site leading to metastasis suppression.

KAI1/CD82 has a quite short half life but in conjunction with other tetraspanins it does increase the shelf life of CD4. This effect is attributed to the combinational formation of tetraspanin web on the cell membrane (Hu et al., 2005). Apart from KAI1/CD82 interactions with other tetraspanins, its binding to cholesterol is also responsible for the normal functioning of cells signalling cascades (Delaguillaumie et al., 2004).

Biological interactions of KAI1/CD82

Effect of KAI1/CD82 in embryo development

Significance of KAI1/CD82 protein during pregnancy was also investigated in 2007 by Gellersen. Trophoblast tissue usually invades the maternal decidua

in a similar fashion to a limited invasion in tumours. Limited invasion of trophoblasts may be attributed to their regulatory mechanism like, formation of multinucleated trophoblastic giant cells which are no longer invasive, induction of apoptosis by tumour necrotic factor release from deciduas cells and release of corticotrophin from both deciduas and trophoblastic cells. These trophoblastic cells were found to be negative for KAI1/CD82 expression in contrast to deciduas layer showing strong KAI1/CD82 expression. Hence tissue specific expression of CD82/KAI1 has also been observed in this study. Strong expression of KAI1/CD82 was in fact modulated by persistent activation of cAMP (Gellersen et al., 2007). This regulation of KAI1/CD82 by cAMP is attributed more towards a post transcriptional level. Knock down of KAI1/CD82 leads to reduced expression of IGFB-1 and decorin (proteoglycan) prompting the role of KAI1/CD82 in differentiation. Decorin inhibits migration by directly binding to EGFR which leads to internalization and degradation. This metastastic inhibitory role of decorin is also strongly correlated with KAI1/CD82 protein (Eshchenko et al., 2007).

Role of KAI1/CD82 in virus infection

Recently, it has been established that virus attachment with tetraspanin web play a significant vital role for many enveloped virus entry and virus release from the cells (Halasz et al., 2005; Martin et al., 2005). Co-expression of Human T-lymph tropic virus (HTLV) glycoprotein and KAI1/CD82 resulted in marked inhibition of syncytium formation. It is noteworthy that CD9 is playing a role in inhibiting Canine distemper virus (CDV) and Feline Immunodeficiency virus (FIV) serving as a model for Human Immunodeficiency virus (HIV) (Willet et al., 1997; Mazurov et al., 2006). Association of tetraspanin with human immunodeficiency virus have been reported earlier (Mathews et al., 2003) but their cellular location along with its potential importance in the budding of virus from macrophage cell membrane has been explored in 2007 by Welsch. In a recent study on HTLV association with TREM, it has been reported that the small inner loop and

Table. 1. Association of KAI1 in clinical perspective with different types of cancers.

Types of cancer	Feature observed with KAI1 molecule	References
Prostate Cancer	Decreased level of KAI1/CD82 transcript in tumour tissue as compared to normal tissue. No correlation of over expression of p53 with down regulation of KAI1/CD82 was observed	Jackson et al., 2003; Cho et al., 2006
Breast Cancer	Down regulation of KAI1/CD82 in mammary tumour progression and its role in brain metastasis. No association with any clinical parameter observed	Yang et al., 2000; Huang et al., 2005; Son et al.,2005; Stark et al., 2005
Lung Cancer	Correlation of FAK and KAI1/CD82 along with MRP-1 Involvement of KAI1 with Clinical parameters and its role as prognostic marker	Nagira et al., 1994; Guo et al., 2005; Liu et al., 2005; Wang et al., 2005
Pancreatic Cancer	Clinical correlation of KAI1/CD82 with increased survival rate in patients showing positive KAI1/CD82 expression	Friess et al., 1998; Guo et al., 2005
Urinary system related Cancer	Decreased expression of KAI1/CD82 leading to differentiation, infiltration and metastasis in urothelial cells. No correlation of alternative spliced form of mRNA with clinical parameters	Su et al., 2004; Jackson et al., 2007; Yuan et al., 2008
Oral/oesophageal squamous cell carcinoma	Down regulation of CD82/KAI1 seen in 73.7% cases. Lack of any correlation with p53 expression or clinicopathological parameters. No mutation on coding region was observed indicating the involvement of other regulatory pathways.	Farhadieh et al., 2004 Miyazaki et al., 2005
Penile squamous cell carcinoma	Inverse correlation with tumour grade and metastasis and patients survival rate	Protzel et al., 2008
Laryngeal carcinoma	Significant relationship between down-regulation of KAI1/CD82 expression with grade and TNM staging was observed	Wu et al., 2003
Gall bladder carcinoma	Inverse correlation of KAI1/CD82 protein and clinical stage of cancer	Jiang et al., 2006
Thyroid cancer	Reduced level of KAI1/CD82 was reported in clinically detected node metastasis in comparison to clinically undetectable cases	Chen et al., 2004; Ito et al., 2005; Kopczynska et al., 2007
Oestosacrocoma	No significant correlation between KAI1 expression and cancer progression has been observed	Leavey et al., 2006
Neuroblastoma	Decrease in expression is an early event in tumour progression	Wu et al., 2005
Digestive system related cancer	KAI1 down regulation at translational scale as a potential prognosis marker in gastric, oral squamous cell carcinoma	Wu et al., 2003; Zheng et al., 2004; Tsutsumi et al., 2005
Ovarian epithelial ovarian cancer	Relationship of tumour grade and metastasis with KAI/CD82. One mis sense mutation present in both tumour and normal cells	Liu et al., 2000; Houle et al., 2002
Cervical cell carcinoma	Involvement in early carcinogenesis and lack of any regulatory role in cancer progression	Schindi et al., 2000
Endometrial carcinoma	Clinical potential of KAI1 as a prognostic marker	Liu et al., 2003

position of cysteine for palmitoylation of KAI1/CD82 molecule are important for its interaction and binding to either T helper or T cytotoxic cells (Mazurov et al., 2007). In Human immunodeficiency virus infection, it was found that Gag protein responsible for viral assembly and release interacts with CD62 on cell surface TERM (tetraspanins enriched micro domain) (Nydegger et al., 2006). CD81 was found to activate T cells and inhibit NK cells after interacting with HCV by changing cytoskeleton actin and due to its degree of association with KAI1/CD82 molecule on cell membrane, similar mechanism has been purposed to be contributed by KAI1/CD82 as well (Crotta et al., 2006). Involvement of dendritic cells (DC) as antigen presenting cell for presenting HIV to CD4 cell also require involvement of CD81, KAI1/CD82 and several other tetraspanin (Garcia et al., 2005). KAI1/CD82, CD9, CD63 and CD81 are playing vital role by down modulating the migratory activity of DC as well (Mantegazza et al., 2004). Hence KAI1/CD82 molecule regulates cytoskeleton structure and is also vital in DC for their antigen presentation to the host.

Effect on cell-cell adhesion

Cell-cell adhesion is an important cellular mechanism in epithelial biology and is a cellular function that restricts cancer cells from departing from each other and disseminating in the body. E-cadherin is one of best investigated cell-cell adhesion molecule in epithelium derived malignancies and is a recognised tumour suppressor molecule. KAI1/CD82 has an ectopic effect on adhesion by strengthening the interactions between E-cadherin and ß-catenin, in doing so; KAI1/CD82 may reduce the chances of cellular dissemination from the primary tumour (Abe et al., 2008). There is a marked correlation among expressional down regulation between E-cadherin and KAI1/CD82 (Wang et al., 2006). Though a marked reduction of KAI1/CD82 expression in retinoblastoma cancer has been previously reported, no significant level of ß-catenin alteration was observed in invasive and non invasive forms, indicating involvement of some other regulatory molecules in the loop (Mohan et al., 2007). Protein of breast cancer antiestrogen resistance gene1 (BCAR1) gene, located on chromosome 16q23, has found been inversely correlated with KAI1/CD82 expression in prostate carcinoma (Fromont et al., 2007). The study measured the level of perturbation made by the loss of 16q23 in hormone therapy replacement. KAI1/CD82 is also responsible for cell homotypic aggregation by Src kinase signalling pathway in prostate cancer cell lines (Jee et al., 2003).

Effect of GSK3b with KAI1/CD82 amyloid and osteoclastogenesis

TCFγ is responsible for the induction of KAI1/CD82 and GSK3ß protein in a study reported on Alzheimer's disease (Vengopal et al., 2007). KAI1/CD82 role as osteoclastogenesis regulator has also been monitored in conjunction with other tetraspanin as CD9, CD37, CD53, CD63, and CD151 (Iwai et al., 2007). Effect of Tspan-5 and Net-6 are antagonist in nature in response to RANKL (receptor activator for nuclear factor κB). Endoplasmic reticulum associated protein Gp78 is responsible for degradation of KAI1/CD82 and inverse relationship between these two molecules is a determinant factor for tumour to either undergo metastasis or become less aggressive (Tsai et al., 2007). This study also points out that the regulation of metastasis by KAI1/CD82 is attributed more towards post-translational down regulation of KAI1/CD82 and is independent of gp78 direct association in metastasis progression. Duffy antigen receptor (DARC) on endothelial cells is chemokines that interact with cancer cells during the extravasation process. It has been shown that DARC is a receptor for those cancer cells which escape from primary tumour and have KAI1proteins on their cell surface (Liizumi et al., 2007). DARC-KAI1/CD82 mediated reaction would lead to the cellular arrest in the vascular system forming a part of 'locking-docking' mechanism during the extravasation (Zijlstra and Quigley, 2006).

Role of KAI1/CD82 on the cellular fate and function of integrins and EGFR

Integrins are key proteins in cell-matrix adhesion and are responsible for mediating various signals to extracellular matrix from the cell surface. They are also responsible for cell shape, mobility and regulation of the cell cycle. Cell traction and migration are mediated by co-ordinated action of integrins and matrix degrading enzymes. Though several types of integrins take part in cell motility, some of them are potentially more potent in cellular motility. For example, α5ß3 and α6ß4 integrins are more actively involved in regulating cellular motility, compared to α5ß1 (Plantefaber and Hynes, 1989). Integrins dependent adhesion with the extracellular matrix is also reduced with over expression of KAI1/CD82 indicating a functional cross talk between them (He et al., 2005). Up regulation of KAI1/CD82 protein is responsible for a marked reduction in invasion and metastatic potential of cancer cells in non small cell lung cancer (Jee et al., 2006) and also stop migration of cells through ß integrin attenuation (Jee et al., 2007). KAI1/CD82 are known to be associated with integrins including α3ß1, α4ß1, α5ß1, α6ß1 and is also involved in the internalization of α6 integrin which acts as a receptor for laminin of the basement membrane. It has also been observed in another study that KAI1/CD82 also regulates the maturation of ß1 integrin (Jee et al., 2007) hence upregulation of KAI1/CD82 protein does not affect the total integrins expression level yet internalization of integrins results in altered cell morphology. Generally, one integrin can bind to more than one tetraspanin, and tetraspanins usually interact

with ß integrins as CD9 association with ß integrins (Yunta and Lazo, 2003). Thus KAI1/CD82, by interacting with and internalising integrins has an inhibitory affect on cellular migration and invasion (He et al., 2005). The other important molecule with which KAI1/CD82 interacts and actively regulates cell function is the cell surface EGF receptor (EGFR). The role of EGF in cancer such as breast cancer has been very well recognised and its receptor is presently a target in cancer therapies. KAI1/CD82 either associates with integrins or EGFR as separate entities on the membrane and around 15-20% of EGFR is co precipitated with KAI1/CD82 molecule (Odintsova et al., 2000). Thus, apart from target inhibition of EGFR either by monoclonal antibodies likes IMC-225 or with the aid of small molecules inhibitors (CI-1033), KAI1/CD82 may also be a promising target for metastasis therapy (Herbst, 2004). KAI1/CD82 was found to induce a classical inhibitory effect on ligand induced dimerization of EGFR and leading to internalization of EGFR ligand (Odintsova et al., 2003) providing cells to senescence its interactions.

Integration of KITENINS

Splice variant of KAI1/CD82 also showed marked reduction in metastasis suppression with alteration of cellular motility and adhesion characteristics. This spliced variant associated specifically with a protein homolog to VANG L1 (Drosophila) in the cytoplasmic region. This VANGL1, termed as KAI COOH-terminal interacting tetraspanin (KITENIN) protein, has been found to act as metastasis promoting protein by increasing invasions and adhesion to fibronectin (Lee et al., 2003, 2004). KITENIN and KAI1/CD82 wild type antagonistic expression can be used as a molecular marker for the prediction of cancer metastasis. In bladder cancer, altered expression of KAI1/CD82 is associated with invasion and in case of KITENIN no such correlation with cancer cells invasion was observed. KITENIN modulates tumour invasion and cellular motility by changing actin cytoskeleton. Marked alteration of actin cytoskeleton is found in Kitenin positive metastatic cell lines (Rowe and Jackson, 2006). In another study, KITENIN has been shown to modulate cancer progression along with 90K and protein kinase C inhibitor PKCI (Lee et al., 2005).

Regulatory role on MMPs and urokinase-type plasminogen

Matrix metalloproteinases are a group of zinc dependent extracellular matrix degrading enzymes. They are responsible for many normal physiological activities like tissue repair, fetus implantation, wound healing, and angiogenesis. Over expression of this family of endopeptidases has been observed in many different types of cancers. These proteins are responsible for degrading collagen fibres of bone or cartilages and extracellular matrix proteins, providing space for cancer cells invasion (Ravanti and Kahari, 2000). Natural antagonists to these matrix metalloproteinase are TIMP (tissue inhibitors of metalloproteinase). They are classified into four protease inhibitors as TIMP1, TIMP2, TIMP3 and TIMP4, all being responsible for inhibiting MMPs by binding Zn ions through their chelating groups (hydroxamates, carboxylates, thiols, and phosphinyls). Increased expression of these TIMPs is associated with a better clinical outcome as observed in a number of tumour types including basal cell cancers (BCC) (Boyd et al., 2008). KAI1/CD82 indirectly regulates the function of MMPs by up regulating TIMP as observed in lung cancer cell lines (Jee et al., 2006). Plasminogen, a precursor of plasmin, upon activation is responsible for breaking fibrin. Activation of plasminogen to plasmin is induced by urokinase plaminogen activators (uPA). These urokinase plasminogen activators (uPA) bind with urokinase plasminogen activator receptor (uPAR) and are involved in mediating cellular adhesion, invasion migration and tissue remodelling. KAI1/CD82 has indirect involvement with urokinase-type plasminogen activator receptor and reduces paracellular proteolytic activity of the enzymes (Bass et al., 2005). It has been observed that KAI1/CD82 increases the binding affinity of uPAR with integrins this lead to marked reduction in uPA mediated cellular cascade (Huai et al., 2006).

KAI/CD82: as prognostic marker of differentiation between metastatic and non metastatic cells

From the function and pattern of molecular interactions of KAI1, it is evident that KAI1/CD82 has an important role to play in the invasiveness and metastasis of cancer cells. Metastasis is a complex cascade process that involves a number of orchestrated events by cancer cells in order to break away from the primary tumours, break down the tissue barriers and break into new organ (secondary site) to form new tumours. As already touched in early sections, KAI1/CD82 is obviously involved in a number of the cellular events which can somewhat be mirrored in clinical studies. Metastasis is influenced by the interaction both tumour and stromal cells like interaction of macrophage with a tumour cell with CSF1 and EGF (Condeelis and Pollard, 2006). Marked reduction of KAI1protein in highly metastatic cancer cells has been observed. Loss or reduced expression of KAI1/CD82 in primary tumours of penile squamous cells showed a positive lymph node metastasis and poor prognosis as compared to lymph node negative and positive expression (Protzel et al., 2008).

KAI1/CD82 reduces matrix invasion by cancer cells

Perhaps one of the most significant impacts of KAI1/CD82 on invasion and metastasis is its influence on the invasion of matrix by cancer cells. Events in matrix invasiveness are cell adhesion to matrix via

integrins, degradation of extracellular matrix by way of proteolytic enzymes including MMPs and high migratory capacity of cancer in order to pass through the degraded matrix. As already discussed, KAI1/CD82 interact with integrins and indirectly influencing TIMP, via which it affects matrix adhesion and degradation, and indirectly impinge on cellular migration and invasion. KAI1/CD82 also interferes with the effects of other invasion promoting factors. KAI1/CD82 reduces the invasions and growth of the cells by inhibiting the functional interaction of integrins with HGF and with ganglioside GM2 (Sridhar and Miranti, 2006; Todeschini et al., 2008). YTS1 cells established from highly metastatic and invasive urinary bladder cancer, lacking in KAI1/CD82, showed marked increased in expression of HGF independent Met tyrosine kinase and cell motility. However, after transfection with KAI1/CD82, reduction of motility and HGF dependent reduction of met tyrosine kinase; due to the formation of CD82/GM2 complex has been observed (Todeschini et al., 2007). Over expression of the CD81 and KAI1/CD82 in myeloma cells reduce the invasion of cells but do not affect on the cell cycle cascade (Tohami et al., 2007). In mouse tumour model, correlation of lung cancer metastasis suppression in regional lymph node was observed after transfection of KAI1/CD82 in mouse (Takeda et al., 2007). Gangliosides are responsible for stability of KAI1/CD82 tetraspanin enriched micro domain formation, its depletion results in weakening of KAI1/CD82 molecular interaction as well (Odintsova et al., 2006). Palmitoylation of KAI1/CD82 on cysteine residues proved a critical step in stopping invasion and migration of cells by inhibiting lamellipodia formation and cytoskeleton actin rearrangement (Zhou et al., 2004).

Induction of apoptosis

KAI1/CD82 was found to induce apoptosis by producing reactive oxygen intermediates (ROIs). Deletion of small and large extracellular loop has no effect on apoptosis, while deletion of third transmembrane domain along with small intracellular loop abrogates apoptotic ability of the cells. Reduced form (90%) of glutathione (GSH) has been observed in normal cells in more abundance than its disulfide form (10%) (GSSG) in normal cells. An increased GSSG-to-GSH ratio is considered indicative of oxidative stress and this reduced form of glutathione enzymes which acts as inhibitor of ROIs observed to be down regulated during KAI1/CD82 induced apoptotic progression. KAI1/CD82 also regulates cdc42 which also plays a role in proapoptotic signalling pathways (Schoenfeld et al., 2004).

The prognostic value of KAI1/CD82 in patients with cancer

Down regulation of the KAI1/CD82 protein has been observed in several cancers. Indirect association of lymph node metastasis with KAI1/CD82 expression has been observed. Its clinical relationships with respect to some of the most recurrent cancers are discussed in the following sections.

Breast cancer

KAI1/CD82 transcript and protein have been found to be reduced or lost in mammary tumours. Decreased expression of KAI1/CD82 has been observed with progression of mammary tumours. In Son's report, although there was no significant correlation found among clinic pathological parameters and KAI1/CD82 molecule, its importance as a prognostic impact has been proposed (Son et al.. 2005) Similarly in another study, it has been observed that both transcript and translational level of KAI1 molecules were reduced in conjunction with KISS1 and MKK4 in invasive mammary ductal carcinoma, an observation linked with brain metastasis (Stark et al., 2005). In a recent study, we have found a significant association of KAI1/CD82 molecule with TNM staging has also been observed in relation to invasive ductal breast cancer patients (Malik et al., 2008a,b).

Urinary system related cancer

Aberrant expression of KAI1/CD82 was also observed in urinary system related cancer. It has been found that level of mRNA of KAI1/CD82 is significantly lower in urothelial cancer as compared to normal bladder tissue. Similarly a strong inverse correlation of KAI1/CD82 protein with tumour grades have been observed highlighting its importance to be used as an affective indicator for differentiation, infiltration and cellular invasion in urinary system related tumours (Yuan et al., 2008). In another recent study, the impact of KAI1/CD82 splice variant in tumour progression has been investigated. The study has shown that out of 20 bladder tumours, 15 were found positive for spliced mRNA form of KAI1. However, there was no association between the presence and absence of AS mRNA and clinicopathological characteristics of these tumours (Jackson et al., 2007). Both studies suggest the potential use of KAI1/CD82 as a prognostic marker in clinical study.

Tumours of digestive system

Decreased expression of KAI1/CD82 protein has been observed in 127 of 174 gastric carcinomas. No significant association with age, gender and tumour location have been observed. The study showed that loss of KAI1/CD82 leads to poor prognosis and KAI1/CD82 also influence on patient's survival. However, KAI1/CD82 alone is not an independent prognostic marker (Wu et al., 2003). Increased overall survival rate was observed in 15 patients with KAI1/CD82 positive

pancreatic carcinoma compared with 25 patients showing a decrease in KAI1/CD82 expression (Sho et al., 1998). Similarly, an inverse correlation of oesophageal squamous cell carcinoma with lymph node metastasis has been observed in 91 patients (Miyazaki et al., 2005). An Inverse correlation between KAI1/CD82 and cancer progression also been reported in gastric cancer (n=113) through immunohistochemical method indicating it substantial involvement in tumour suppression (Zheng et al., 2004).

Gynaecological cancer

In a cohort of 32 primary and 8 metastatic ovarian cancers (n=8), an inverse correlation of KAI1/CD82 with tumour grade and metastasis has been observed (Houle et al., 2002). KAI1/CD82 association in invasive and metastatic lesion of cervical cancers has also been observed but authors do not observe any significant correlation with respect to cancer progression (Liu et al., 2001). In another study with 75 cervical carcinomas, early loss of KAI1/CD82 expression in cancer progression has been reported. No association of KAI1/CD82 with any clinical parameters was observed thus indicating the possible involvement of other mechanisms of genetic regulation (Schindi et al., 2000). Patients with KAI1/CD82 negative endometrial cancers have lower survival rates, thus emphasizing its potential role as a strong prognostic candidate (Liu et al., 2003).

KAI1/CD82 in soft tissue sarcoma

In contrast to previous findings, it has been observed that KAI1/CD82 does not have any statistically significant correlation with clinical parameters in osteosarcoma. These findings are made after screening 46 samples (32 tumour and 12 metastatic tissues) where KAI1/CD82 expression was not significantly different between tumours or metastatic tissues (Leavey et al., 2006). Earlier in a study, no significant correlation of KAI1/CD82 molecule with clinical progression of cancer has been observed in either malignant or benign tumour tissues (Arihiro and Inai, 2001). The tissue specific trend of KAI1/CD82 expression is an area that requires further research and investigation.

Neurological cancer

In an earlier study, complete loss of KAI1/CD82 was observed in 20 out of 30 tumour tissues and very weak expression in the remaining 10 samples. However, there is no significant correlation between KAI1/CD82 and any clinical parameter was observed (Aryee et al., 2002). A recent study has shown that KAI1/CD82 has a stronger staining in ganglioneuroblastoma than in neuroblastoma (Wu et al., 2005)

Lung cancer

In lung cancer, a strong correlation of KAI1/CD82 was observed with tumour differentiation, TNM system, lymph node metastasis and histological types indicating KAI1/CD82 as a potential prognostic marker (Liu et al., 2005). In another study in lung cancer, indirect correlation of KAI1/CD82 with FAK and direct with MRP-1 was observed showing their potential role in invasion and metastatic involvement (Wang et al., 2005). FAK increased expression has been reported with cancer progression. A recent study using in vivo lung tumour model and adenoviral driven KAI1/CD82 delivery, it has been shown that KAI1/CD82 significantly reduced the metastasis to mediastinal lymph nodes (Takeda et al., 2007). Collectively, these studies have shown a potential therapeutic value of KAI1/CD82 in cancers, particularly in lung cancer.

Conclusion

It is clear from the literature review that KAI1/CD82 play an important role in cancer and cancer metastasis. Its inhibitory role in cell motility and invasion as well as cell adhesion, together with the clinical observations that KAI-1 expression is often lost/reduced in cancer, strongly pointed KAI1/CD82 as having a therapeutic role in cancer. Indeed, recent pre-clinical investigation has showed such promise. Despite all these exciting progress, a number of questions remain to be answered with regard to KAI1/CD82 and its role in cancer. How regulation of KAI1/CD82 molecule initiates in cancer cells and whether KAI1/CD82 itself is responsible for its regulation in tumour progression? Is KAI1 alone is sufficient enough to induce metastasis suppression at its own or act as a molecular facilitator in various signalling pathways? How we can induce an artificial but sustainable expression of this molecule in the human body? What effect may be observed in sustainable KAI1 expression in human suffering from various types of tumours? Anwers to these questions will have significant impact on the understanding of the biology of KAI1/CD82 in cancer.

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