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Current evidence highlights the ability of adaptor (or scaffold) proteins to create signalling platforms that drive cellular transformation upon integrin-dependent adhesion and growth factor receptor activation. The understanding of the biological effects regulated by these adaptors in tumours might be crucial for the identification of novel targets and the development of innovative therapeutic strategies for human cancer. In this review we will discuss the relevance of adaptor proteins in signalling originating from integrin-mediated cell-extracellular matrix (ECM) adhesion and growth factor stimulation within the context of cell transformation and tumour progression. Herein, we will specifically underline the contribution of p130CAS, NEDD9, CRK, and the IPP complex (ILK, PINCH and PARVIN) to cancer, along with the more recently identified p140CAP.

Introduction

In the last fifteen years integrin signalling has been profoundly implicated in cancer cell proliferation, survival and invasion[1]. Current knowledge implies that in cancer cells integrins, receptor tyrosine kinases (RTKs) and cytokine receptors constitute joint modules in which attachment to the ECM confers positional control to the activated signalling pathways allowing cells to respond to soluble growth factors, which thereby determine the nature and the extent of the response[1,2]. This control mainly resides on the recruitment of adaptor proteins that behave as molecular hubs for intracellular signalling and organise complex signalling networks in time and space[3-6]. The biological effects regulated by integrin and RTK signalling adaptors are dependent on their expression levels and on their phosphorylation status, which determines the association with binding effectors.

The integrin signalling adaptors represent crucial players in cell transformation and invasion, mostly by regulating basic processes such as cell cycle control, survival, cytoskeletal re-organisation and migration. Recent studies highlight the relevance of specific families of these scaffold molecules in many human cancers and show that interfering with their expression and/or

with their ability to bind effector proteins is therapeutically efficacious to inhibit tumorigenesis sustained by integrin and growth factor receptor co-operation. This Review will discuss how the expression of integrin adaptors is related to human cancer, their relevance in animal models of tumorigenesis and their role in cancer cell biology.

Integrin adaptors in cancer

In the following paragraphs we will review recent data that highlight how the altered expression of the integrin adaptors is related to tumorigenesis. p130CAS, NEDD9, CRK, the IPP complex (ILK, PINCH and PARVIN) and p140CAP adaptors are crucial effectors of integrin and RTKs signalling, acting as multi-site scaffolds that integrate and propagate signals from ECM and soluble ligands to intracellular signalling pathways, promoting cell proliferation, survival and motility.

The CAS family. The CAS family comprises four members: p130CAS (also known as breast cancer anti-oestrogen resistance 1 (BCAR1)), NEDD9 (also known as HEF1 or CAS-L), embryonal Fyn-associated substrate (EFS, also known as SIN) and CAS scaffolding protein family member 4 (CASS4, also known as HEPL). They are characterized by the presence of multiple conserved sequence motifs, such as SH3 and proline-rich domains and extensive post-translational modifications, mainly consisting of tyrosine and serine phosphorylation[3,4] (Figure 1). CAS proteins differ in patterns of expression, for esample in normal tissues, whilst p130CAS is ubiquitously expressed, NEDD9 expression is confined to the lungs and kidneys[3,4]. CASS4[7] and EFS[8] are less abundant and specifically expressed in spleen and lung (CASS4) and T- lymphocytes, thymus, brain and skeletal tissue (EFS), making their functions limited to specific context.

Overexpression of CAS proteins contributes to the development of human cancer. p130CAS is necessary for transformation by several oncogenes, such as SRC, ERBB2 (also known as HER2) and nucleophosmin (NPM)-anaplastic lymphoma kinase (ALK) fusion protein[9-11]. Recently, p130CAS has been shown to be required for *KRAS*, *BRAF*, *PTEN* and *PIK3CA* oncogene-dependent proliferation[12]. Nevertheless, investigation of its expression in biopsies of different human malignancies using immunohistochemistry is still limited to breast cancer and haematological malignancies (Table I). Recently it has been reported that in human breast cancers overexpression of both ERBB2 and p130CAS is associated with increased proliferation, metastasis formation and poor prognosis[13,14](Table I). Consistently, double transgenic mice overexpressing both p130CAS and ERBB2 in the mammary gland show an accelerated onset of tumour formation, providing evidence that p130CAS and the ERBB2 oncogene synergise *in vivo* to transform the mammary epithelium[13] (Table II). Indeed p130CAS silencing in ERBB2 transformed breast cancer cells is

sufficient to inhibit tumour growth *in vivo*, and correlates with downregulation of proliferative and survival pathways, such as SRC and AKT activation, focal adhesion kinase (FAK) phosphorylation and CYCLIN D1 expression[11]. In oestrogen receptor (ER)-positive human breast tumours, overexpression of p130CAS correlates with intrinsic resistance to tamoxifen treatment in a large subset of human breast cancer samples[15-17].

The second member of the family, *NEDD9*, has been identified as a metastasis gene in melanoma and in head and neck squamous cell carcinoma (HNSCC). Indeed its expression levels is elevated in human metastatic melanoma compared with primary melanoma[18,19] and in invasive HNSCC[20]. Recently, a role in mammary tumorigenesis has also been proposed for NEDD9, whose absence significantly impairs tumour formation induced by the polyoma virus middle T oncogene (PyMT)[21] (Table II).

Overall, p130CAS and NEDD9 expression levels are critical for onset and progression of many aggressive cancers, highlighting their importance as new unfavourable prognostic markers.

The CRK family. The CRK family consists of three members: CRK-I, CRK-II[22], alternative transcripts of the same gene, and CRKL[23] (Figure 1). The CRK family SH2 domains can bind to a variety of key signalling molecules including p130CAS.

The CRK family has been shown to be overexpressed in lung adenocarcinoma[24], human colon cancers[25] and malignant glioblastoma[26,27]. High levels of *CRK* mRNA and protein expression correlate with increased tumour aggressiveness in lung, contributing to poor prognosis and shorter survival[24]. Recently, it was shown that CRK is a key regulator of mammary gland tumorigenesis. A subset of mouse mammary tumour virus (MMTV)-*CRK* transgenic mice develop focal mammary tumours with a latency of 15 months, suggesting a potential role of CRK in integrating signals for breast cancer progression *in vivo*[28] (Table II).

The IPP complex. The IPP adaptor complex comprises the integrin-linked kinase (ILK), PINCH1 (also known as LIM and senescent cell antigen-like domains 1 (LIMS1)) and PINCH2 (also known as LIMS2) and PARVIN α, β and γ (Figure 1).

The expression of ILK has been analysed in a large number of human malignancies[29-31] and is often found to be elevated and associated with tumour progression and shortened survival (Table 1). Increased ILK expression has been associated with more differentiated areas of malignant gastrointestinal, renal, neural and bone marrow tumours, suggesting that ILK might be also an indicator of differentiation[29,32].

Evidence for the role of ILK in breast cancer development derives from the generation of transgenic mice overexpressing ILK in the mammary gland epithelium, where ILK overexpression leads to mammary gland hyperplasia and breast tumours of diverse phenotypes[33]. Moreover, targeted ablation of ILK in the mammary gland provides a direct demonstration that this molecule is required in the initiation phase of ERBB2-induced tumours, resulting in delayed tumour growth *in vivo* and a profound block of invasive properties *in vitro* due to the induction of apoptotic cell death[34]. Recently, it has been described that transgenic MMTV-Wnt/ILK mice show lobuloalveolar hyperplasia and significant acceleration in mammary tumour incidence and growth[35] (Table II).

Less is known about the expression of the other members of the IPP complex in cancer tissues. *PARVB* (which encodes PARVIN β) mRNA and protein levels are markedly down-regulated in a number of advanced breast tumours[36] and its expression is inversely correlated with ILK protein and kinase activity levels, suggesting that down-regulation of PARVB expression upregulates ILK activity in human tumours (Table 1). Regarding PINCH1/2 expression in samples of breast, prostate, lung, colon, and skin carcinomas, both proteins have been found to be up-regulated in tumour-associated stromal cells, especially at the tumour invasive edges[37]. In particular, in a large series of colon cancers, strong PINCH1/2 stromal expression was associated with a lower patient survival, placing these molecules as independent prognostic indicators of colon cancer[38](Table 1).

The CAP family. The CAS-associated protein (Cap) family consists of two members, p140CAP (also known as SRC kinase signalling inhibitor 1 (SRCIN1) or SNIP) and SICKLE TAIL (SKT, also known as KIAA1217). p140CAP has been shown to down-regulate integrin and growth factor-dependent signalling[39,40]. p140CAP[41,42] and SKT[43,44] are multisite docking proteins, characterized by conserved sequence motifs that can associate with multiple effectors (Figure 1).

p140CAP is mainly expressed in brain, testes and epithelial-rich tissues such as mammary glands, lungs, colon and kidneys[41,45,46] and is phosphorylated on serine and tyrosine residues[41,46,47], whose relevance in downstream signalling needs to be assessed. p140CAP behaves as a tumour suppressor protein, since its silencing favours anchorage-independent growth and *in vivo* tumour growth[39,40]. Although few data are available on p140CAP expression in human tumours, a recent screening of mammary breast cancers revealed that p140CAP is not expressed in 70% of tumour specimens characterised by G3 index, node positivity and a high proliferation index, thus indicating an inverse correlation with the state of malignancy[39]. By contrast, an additional report shows that *SRCIN1* mRNA expression positively correlates with unfavourable prognostic factors in breast cancer[48]. These apparently conflicting results suggest the existence of DNA mutations or

chromosomal rearrangements that affect protein translation, resulting in reduced levels of p140CAP expression in tumours. Indeed, *SRCIN1* is located on human chromosome 17q12 and its flanking regions contain several genes involved in tumour initiation and progression such as *ERBB2* (17q12), *BRCA1* (17q21), retinoic acid receptor α (*RARA*, 17q21) and signal transducer and activator of transcription 3 (*STAT3*, 17q21), which are often translocated as fusion partners of the mixed-lineage leukaemia (*MLL*) gene in haematological malignancies, or amplified in human tumours, suggesting that *SRCIN1* may also be subjected to such chromosomal rearrangements[49].

Integrin adaptors in cancer biology

The data summarised above clearly point out that the expression of integrin adaptors is deregulated in a variety of human cancers. Although the precise molecular mechanisms implicated in this altered expression are currently not understood, extensive work on cancer cell models show that these scaffold proteins are involved in cancer initiation, progression and metastasis formation. Thus, their involvement in these three fundamental steps of tumorigenesis, makes them attractive targets for prognostic and therapeutic purposes.

The CAS family in cancer biology

The CAS family represents a nodal signalling platform on which integrin and growth factor receptor signalling convey. As a consequence, they are implicated in key events in cancer cell biology such as the acquirement of pro-survival and pro-invasive phenotypes. The molecular mechanisms underlining these events will be discussed in this section.

Migration and invasion. In cancer progression, cell-cell detachment from the primary tumour and the acquirement of a motile phenotype are required for cells to become invasive and colonise distant organs to generate metastasis. Growing evidence shows that CAS family proteins are major players in migration, invasion and metastasis formation, mainly by undergoing phosphorylation, activating RHO family GTPases and inducing metalloproteinase gene expression.

Tyrosine phosphorylation of the p130CAS substrate domain (SD) mainly by SRC and FAK[50] allows the assembly of the p130CAS-CRK- DOCK1 (dedicator of cytokinesis 1, also known as DOCK180) complex and efficient localized activation of the small GTPase RAC1 at the cell membrane. These events induce cell migration through actin cytoskeleton remodelling, pseudopodia extension and focal adhesion turnover[51-53]. Uncoupling of p130CAS-CRK negatively regulates cell migration. Indeed, the non-receptor tyrosine kinase ABL1 phosphorylates CRK-II on tyrosine 221 (Figure 1), inducing intramolecular folding that prevents binding of the C-terminal

CRK-II SH2 domain to the phosphorylated SD of p130CAS, leading to decreased cell movement[54,55].

p130CAS has also emerged as a pro-invasive molecule. To regulate invasion p130CAS associates with the ZYXIN family members (ZYXIN, AJUBA, also known as JUB, and thyroid hormone receptor interactor 6 (TRIP6)[56-58] and the transcription factor zinc finger protein 384 (ZNF384, also known as CIZ or NMP4)[56]. These interactions lead to transcription of matrix metalloproteinase genes required for the invasive program[59].

In the context of SRC transformation, SRC requires p130CAS for organization of actin into podosomes, activation of matrix metalloproteinase 2 (MMP2), and the formation of lung metastases *in vivo*[60]. Moreover, bosutinib, a novel SRC kinase inhibitor, has been reported to inhibit breast cancer cell migration and invasion by affecting the SRC-FAK-p130CAS signalling pathway[61].

In ERBB2-transformed cells, p130CAS overexpression confers invasive properties in 3D cultures, sustaining and strengthening PI3K/AKT and ERK1/2/p70S6K signalling downstream of ERBB2, which led to RAC1 activation and MMP9 secretion[11], respectively[14]. Consistently, p130CAS silencing in ERBB2-dependent breast carcinoma impairs migration and invasion *in vitro* as well as the formation of lung metastases *in vivo*[11].

It has been recently proposed that ECM stiffness modifies the context of signaling and promote invasion of oncogene-transformed pre-malignant mammary cells[62] and that integrins are mechanosensors for matrix and tissue rigidity[63]. Interestingly, p130CAS has been described as a major mechanotransduction protein, that undergoes phosphorylation upon force-mediated conformational changes[64] and senses fibronectin, but not collagen, rigidity[65]. These data suggest that p130CAS may strenghten the intracellular signalling cascades activated by tumour-enhanced ECM stiffness.

The role of NEDD9 as a pro-migratory gene has been determined based on its ability to activate RAC1 in highly metastatic melanomas, by forming a complex with DOCK3, a RAC1 GEF. RAC1 activation results in switching from amoeboid to mesenchymal movement, suppression of Rho GTPase activation and loss of actomyosin contractility and motility[66]. Also in glioblastoma cells, NEDD9 is pro-migratory upon platelet-derived growth factor (PDGF)-stimulation[67]. Opposite effects have been reported in a recent siRNA screen of human mammary epithelial cells. In this report, NEDD9 has been classified as anti-migratory, probably through its ability to polarize the microtubule network, suggesting a role for NEDD9 in MTOC (Microtubule-Organising Center) polarization[68]. Further investigations are required to reconcile these opposite data, that might be explained by the different cellular context analysed.

TGFβ signalling and cancer progression. A role for p130CAS and NEDD9 in transforming growth factor-β (TGFβ) signalling has recently been proposed. This regulatory cytokine exerts tumour-suppressive effects that cancer cells must elude for malignant evolution. Yet, paradoxically, cancer cells elicit mechanisms that subvert the tumour suppressing functions of TGF-β, and in doing so, confer oncogenic and metastatic activities upon this multifunctional cytokine[69,70]. In epithelial cells, integrin β1 suppresses apoptosis and growth inhibition induced by TGFβ[71]. In this context p130CAS has been shown to be a crucial player by binding to SMAD3, and preventing its phosphorylation by TGFβ receptor. As a consequence, the transcription of the cyclin-dependent kinase inhibitors p15 and p21 is inhibited, resulting in cell cycle progression[72]. Recently, it has been reported that p130CAS over-expression in mammary epithelial cells (MECs) shifts TGFβ signalling from SMAD2/SMAD3 phosphorylation to p38 MAPK activation, rendering MECs resistant to TGFβ-induced growth arrest and enhancing their metastatic potential[73]. Notably, NEDD9 was found to be a new transcriptional target of TGFβ signalling in highly metastatic mammary adenocarcinoma cells that switch from collective motility to single cell motility, and its silencing results in inhibition of amoeboid motility in response to TGFβ[74]. Overall, CAS family can act as a molecular rheostat that switches the tumour suppressor function of TGFβ to a pro-metastatic role during breast cancer progression.

Epithelial-Mesenchymal transition (EMT) is a known mechanism through which epithelial cells lose their epithelial cell characteristics to acquire a mesenchymal phenotype and become migratory and invasive[68]. ECM molecules such as collagen I have been shown to induce EMT transition in transformed epithelial cells[75]. Highly invasive pancreatic cancer cells bind to collagen I through integrin β1 and discoidin receptors, and up-regulate N-cadherin on the cell surface, which leads to increased cell motility[76,77]. In this model, p130CAS plays a crucial role in collagen I–mediated cell movement as well as in the up-regulation of N-cadherin, by acting as a scaffold, integrating integrin β1 and discoidin receptors in the plasma membrane[78]. These findings place the CAS family as potential modulators of cell matrix-dependent EMT for the acquirement of aggressive cancer properties.

Apoptosis. Cell death by apoptosis serves as a protective mechanism to maintain cell homeostasis in the adult organism and its inhibition contributes to transformation. p130CAS and NEDD9 are both targets of apoptotic pathways, undergoing proteolytic cleavage and generating fragments that directly participate in the disruption of focal adhesions and cell death. Caspase 3-dependent cleavage of p130CAS releases a 31kDa fragment that has pro-apoptotic activities. This fragment translocates to the nucleus and heterodimerises with the transcription factors E2A (also known as

TCF3) or E47, thereby repressing p21transcription, promoting loss of focal adhesions and inducing cell rounding and cell death[79,80]. Similarly, the proteolytic cleavage of NEDD9 by caspases 3 and/or 7 releases a C-terminal p28 fragment, that in MCF-7 and HeLa cells induces focal adhesion disassembly, cell detachment and apoptosis[81], in a mechanism similar to that described for p130CAS, implicating binding to E2A and transcriptional repression of p21.

In tumours, p130CAS cleavage might represent a mechanism to interfere with cancer cell survival. It has been observed that silencing of the protein tyrosine phosphatase regenerating liver (PRL-3) leads to induction of p130CAS cleavage and anoikis, preventing anchorage-independent growth of colon carcinoma cell lines[82]. Interestingly, upon PRL family knock-down in colon and lung cancer cells[82,83], p130CAS expression is reduced, indicating that these phosphatases are crucial regulators of p130CAS protein levels. In addition, it has been described that p130CAS cleavage may result from over-expression of the chemokine CXCL12[84] and also from the inhibition of activity of cyclooxygenase 2 (COX2), a key enzyme in prostaglandin synthesis that promotes tumour progression and angiogenesis[85]. Treatment of colon cancer cells with the COX2 inhibitor celecoxib induces proteolysis of p130CAS and nuclear translocation of the 31 kDa fragment, which leads to apoptosis[86]. Moreover, in a panel of human acute myeloid leukemia cell lines, the anti-tumour effect observed upon treatment with celecoxib is due to the inhibition of p130CAS signalling leading to apoptosis[87].

In conclusion, CAS family behaves as crucial scaffolds to modulate cell survival of a variety of cancer cells. Therefore, interfering with the pro-survival properties of the CAS adaptors might represent an important mechanism to trigger cancer cell death.

The CRK family in cancer biology

The ability of the CRK family of adaptor proteins to induce cell transformation, migration and invasion mainly relies on the p130CAS-NEDD9 and the FAK-SRC pathways, making CRK proteins an attractive target due to its central integrative downstream role in signalling by these molecules[5]. v-CRK-mediated transformation induces elevation of a p130CAS-associated activity of a SRC family kinase member (SFK)[88], FAK phosphorylation by SFK and the recruitment of PI3K to FAK[89,90]. Overexpression of CRK in tumour cells leads to an increase in p130CAS tyrosine phosphorylation and the activation of an intracellular feedback loop that further increases CRK activity and induces motility and the aggressive potential of cancer cells. Therefore, CRK family proteins are not simply conduits for intracellular signal transduction but can also control the amplitude of signalling.

Migration and invasion. In addition to the role of CRK in CAS family-mediated migration and invasion, in synovial sarcoma cells lines CRK has a crucial role in response to hepatocyte growth factor (HGF) by leading to activation of RAC1 through the sustained binding of CRK to GRB2-associated binding protein 1 (GAB1), a docking protein that upon c-Met phosphorylation recruits CRK proteins. CRK silencing remarkably suppresses tumour formation in human synovial sarcoma cell xenografts and invasive growth *in vivo*[91,92]. In glioblastoma cells, CRK over-expression increases cell migration and invasion, likely through an association with DOCK180[26] and its silencing suppresses early attachment to laminin, cell motility and growth[27]. Recent studies show that CRK is down-regulated by miR-126 microRNA in lung cancer cells resulting in impaired cell adhesion, migration and invasion[93]. It is still an open issue as to what regulates the assembly of specific CRK complexes in different tumour cells.

The IPP complex (ILK/PINCH and PARVIN) and cancer biology

The IPP complex has emerged as an essential constituent of integrin containing adhesion sites and it can function both as a structural module that connects integrins to actin cytoskeleton and as a signalling platform that modulates a variety of cellular processes. It is known that IPP members expression in physiological conditions controls normal development and tissue homeostasis. On the other hand, correlative studies of the three IPP members in cancer biology are still lacking and only the single components have been analysed[6,94].

The ILK protein is a central component for the assembly of the IPP complex, where it contributes both with its kinase activity and its adaptor features[95-98]. Indeed, ILK adaptor function is required for binding to PARVIN α and PARVIN γ, but while PARVIN α inhibits ILK kinase activity[99], PARVIN γ association results in induction of ILK kinase activity[98]. Therefore, the degree of ILK kinase activity within the IPP complex might be modulated by its association with different binding partners depending on specific cellular cues. The regulation of the kinase activity by the assembly of different complexes might also explain the observed role of ILK as an oncogene or a tumor suppressor as outlined in the sections below.

ILK as on oncogene. ILK has been described to be involved in all aspects of cancer cell progression. ILK overexpression in epithelial cells leads to cell transformation, invasion, and acquisition of survival and mesenchymal properties, supporting a role for ILK as an oncogene[31]. In mammary epithelial cells, ILK overexpression triggers EMT, characterized by loss of E-CADHERIN and β-CATENIN in adherence junctions and β-CATENIN accumulation in the nucleus, leading to increased synthesis and deposition of fibronectin, further supporting the ILK-dependent EMT[100]. ILK transformed cells undergoing EMT are characterized by increased

migration through activation of small GTPases RAC and CDC42 whose activity is inhibited by ILK knockdown or suppression of its kinase activity[101]. It has also been observed that ILK over-expression in intestinal and mammary epithelial cells leads to a highly invasive phenotype resulting from MMP9 up-regulation and activation[102].

Recently, a new function of ILK in the regulation of the microtubule cytoskeleton and mitotic spindle organization has been proposed[103]. Inhibition of ILK expression or activity impairs centrosome clustering in several breast and prostate cancer cell lines with centrosome amplification and induces mitotic arrest and cell death, demonstrating that inhibiting ILK offers a selective means of targeting cancer cells[104].

ILK as a tumor suppressor. ILK has also been described as a tumour suppressor in cancer cells. For example, ILK expression levels are higher in MCF10A breast epithelial cells compared to breast carcinoma cell lines, suggesting that ILK is lost during breast epithelial cell transformation[105,106]. A rational for explaining ILK oncogenic and tumour suppressing functions in tumour models has been recently established in aggressive paediatric rhabdomyosarcoma (RMS) tumours[107]. RMS tumours largely belong either to embryonic RMS (ERMS), or alveolar RMS (ARMS) histology[108]. Herein, *in vivo* and *in vitro* studies indicate that in ERMS, endogenous ILK suppresses phosphorylation and activation of JNK1/c-JUN signalling, causing growth reduction and induction of apoptosis. On the other hand, in ARMS, endogenous ILK induces JNK phosphorylation, resulting in enhanced growth. Therefore, this work might provide a mechanistic insight into the role of ILK in RMS, as well as in other tumours, suggesting that patients should be stratified on the basis of ILK activity and JNK1 expression, in order to determine which patients may benefit from ILK inhibition as a form of targeted anti-tumour therapy[107].

PARVINS and PINCH in cancer biology. While PARVIN α has been shown to promote anti-apoptotic signalling as a downstream element in ILK signalling,[95,109], PARVIN β behaves as a tumour suppressor, by inhibition of ILK signalling. Consistently, in breast cancer cells, PARVIN β mRNA and protein levels are markedly down-regulated in a number of advanced tumours, and also in certain cancer cell lines, together with increases in ILK protein and kinase level activity[36]. High levels of PARVIN β correlate with increased cell adhesion, induce reversal of anchorage-independent growth[36], and attenuation of tumour growth in nude mice[110]. The latter tumour-suppressive role of PARVIN β is consistent with deletions observed in its locus (22q13.21) in some colon and breast cancer cells[111]. Consistently, several lines of evidence have implicated PINCH1/2 as suppressors of apoptosis. For example, *in vitro* inhibition of PINCH1 expression in HeLa cervical, fibrosarcoma, breast, prostate, hepatocellular, lung, and colon carcinomas leads to apoptosis[109,112]. *In vivo*, knockout of PINCH1 in embryonic neural crest cells caused enhanced

apoptosis[113].

The Cap family in cancer biology

The major function of the p140CAP adaptor in tumour cells is to regulate SRC kinase activation[39,40]. In particular, in breast cancer cells, upon cell-ECM adhesion or epidermal growth factor (EGF) stimulation, p140CAP activates CSK kinase[40], which by phosphorylating the inhibitory tyrosine on the C-terminal domain of SRC, allows the closure of SRC in an inactive conformation[114] (Figure 4a). Therefore p140CAP represents a new potent regulator of the proto-oncogene *SRC* that is able to shift the balance between active or inactive SRC. Consequently, integrin signalling dependent on SRC, such as tyrosine phosphorylation of FAK and p130CAS and RAC1 activation are impaired in cells expressing high levels of p140CAP [40].

In tumour cells p140CAP also regulates E-CADHERIN-dependent cell-cell adhesion. p140CAP regulates cell-cell contact dynamics by increasing the amount of immobilized E-CADHERIN at the cell surface, thereby regulating the strength of cell-cell adhesion. This mechanism also depends on the inhibition of SRC kinase activity[39]. E-CADHERIN is known to inhibit EGF receptor (EGFR) signalling, either by interaction through the extracellular domains or by a β-CATENIN-dependent mechanism[115-117]. Indeed EGFR, RAS and ERK1/2-MAPK activities are profoundly impaired when p140CAP is overexpressed and enhanced when it is silenced[39]. p140CAP also regulates the RAS pathway through an additional unknown mechanism[39]. Therefore, in cancer cells, p140CAP regulates EGFR signalling through a dual mechanism, involving E-CADHERIN-dependent inactivation of EGFR and a RAS-dependent inhibition of ERK1/2-MAPK activity.

For the second member of the family, SKT, one report in prostate cancer cells shows that *KIAA1217* (which encodes SKT) expression is repressed by the androgen receptor, suggesting a potential role for SKT as a tumour-suppressor gene whose loss may contribute to prostate carcinoma[118].

Migration and invasion. p140CAP decreases the ability of breast cancer cells to spread on ECM proteins and to migrate and invade in *in vitro* assays. Consistently, p140CAP silencing accelerates the early phases of cell spreading on ECM, induces a fibroblastic-like morphology and increases motility and invasion[40]. In addition, p140CAP specifically interferes with the ability of both breast and colon cancer cells to scatter from a compact colony in response to EGF[39]. The mechanism by which p140CAP interferes with cell scatter is based on its ability of p140CAP to immobilize E-CADHERIN at the cell membrane as described above.

Actin cytoskeleton remodelling is a crucial requirement for cell scatter and motility. p140CAP has been shown to co-localize with actin stress fibres and cortical actin and to associate with proteins involved in actin cytoskeleton dynamics, such as p130CAS, SRC, VINEXIN and CORTACTIN[41,42,119]. These findings along with the presence of a putative actin-binding domain in p140CAP (Figure 1), suggest that this adaptor could be directly or indirectly involved in actin filaments assembly. Whether other p140CAP-binding proteins are also involved in regulating actin cytoskeleton organisation and cell motility remains to be investigated.

Proliferation. In addition to cell motility and invasion, the ability of p140CAP to regulate SRC and RAS pathways also profoundly affects cell proliferation. Elevated expression of p140CAP in both breast and colon cancer cells inhibits proliferation *in vitro*, but does not affect cell survival[39,40]. Interestingly, in breast cancer cells, p140CAP expression controls anchorage-independent growth, probably by inhibiting downstream integrin signalling, such as SRC and RAC1 activation[40]. Moreover, in breast and colon cancer cell xenografts high levels of p140CAP impair tumour formation[39,40]. Consistently, xenografts of p140CAP silenced carcinoma cells dramatically increases tumour formation *in vivo*[39,40]. Strikingly, p140CAP knock-down is sufficient for *in vivo* growth of oestrogen-dependent MCF7 breast cancer cells even in the absence of oestrogen pellets, a condition in which control cells are unable to grow. These last findings also raise the possibility that p140CAP may regulate oestrogen receptor signalling, contributing to breast cancer resistance to hormonal therapies. In conclusion, p140CAP behaves as a tumour suppressor protein in breast and colon cancer cells, with a broad effect on cell proliferation and tumour growth.

Clinical implications of integrin adaptors

Growing evidence supports a central role for p130CAS in the acquirement of resistance to breast cancer therapy. In oestrogen receptor positive breast cancer cells, p130CAS enhances oestrogen-dependent cell cycle progression, by associating with oestrogen receptor α, SRC kinase and the p85 subunit of PI3K[120]. *BCAR1*, which encodes p130CAS, is upregulated in breast tumours resistant to tamoxifen[121,122]. Consistently, the over-expression of p130CAS in breast cancer cells induces proliferative signals that are not sensitive to treatment with either tamoxifen or oestrogen receptor antagonist fulvestrant[121,123]. Although it has been reported that a possible mechanism through which p130CAS induces tamoxifen resistance is its binding to BCAR3/AND34, a putative GEF for RALA, RAP, and RRAS GTPases[124], the exact mechanism by which p130CAS and BCAR3 induce anti-oestrogen resistance remains unclear. In addition, it has been suggested that both serine and tyrosine phosphorylation of p130CAS are required for mediating oestrogen resistance[125,126].

Recently, high levels of p130CAS in human breast cancer have also been associated with resistance to the cytotoxic agent doxorubicin. In particular, p130CAS-dependent SRC, AKT, ERK1/2-MAPK activation and inhibition of apoptosis are thought to be crucial to confer resistance to this cytotoxic agent[127].

Although displaying highly conserved structural features with p130CAS, NEDD9 does not correlate with oestrogen resistance in breast cancer[126]. However, NEDD9 is overexpressed in imatinib-resistant gastrointestinal stromal tumour cells[128], suggesting that over-expression of a specific CAS family adaptor is selective for the acquirement of resistance to therapy in different cancer subtypes.

We can argue that p130CAS and NEDD9 confer resistance to cancer therapy by clustering and simultaneously amplifying several signalling pathways involved in cell proliferation and survival, thus rendering cancer therapy not effective. It is possible that also the other integrin adaptors might mediate resistance to therapy through similar mechanisms.

The involvement of integrin adaptors in tumour initiation and progression and the association with acquired resistance make them suitable targets for cancer therapy. Strategies for targeting these adaptors may represent a novel and intriguing approach to interfere with tumorigenesis. Silencing of p130CAS, NEDD9, CRK and ILK has provided new insights for the therapeutical use of these adaptors molecules. Interestingly, injection of *BCAR1*-specific siRNAs in the mammary gland of transgenic mice harbouring ERBB2-dependent spontaneous adenocarcinoma was sufficient to inhibit signalling and to reduce tumour initiation [11]. This study represents the first example of a preclinical study using *BCAR1* siRNA in the treatment of ERBB2-dependent breast tumours and indicates that p130CAS might be a potential therapeutic target for ERBB2-dependent tumours.

There is now considerable evidence that ILK can be successfully targeted to inhibit tumour progression in clinically relevant models of cancer. The use of RNAi and antisense oligonucleotides to downregulate ILK expression and small molecule inhibitors to abrogate its kinase activity, has been shown to reverse ILK oncogenic effects in a variety of cancers. Indeed inhibition of either ILK expression or its activity results in decreased cell migration and invasion, metastasis formation, induction of apoptosis *in vitro*[30,129,130] and *in vivo*[131-133]. In addition, on the basis of the new role of ILK in mitosis[103,104], the potential targeting of ILK as an anti-mitotic chemotherapeutic might provide a promising alternative to the chemotherapeutics avoiding severe toxic side effects and drug resistance.

Regarding p140CAP and its potential role as tumour suppressor, the generation of decoy proteins that could function as the intact protein into cancer cells, may have significant potential

therapeutic applications. Moreover, human breast cancer analysis shows that p140Cap expression is inversely correlated with the aggressiveness of malignancy, suggesting that p140Cap can be applied in routine diagnosis as a new prognostic factor recognizing low aggressive breast tumours.

Structure-based design of inhibitors should also be developed to target specific domains of these adaptor proteins, in particular regions containing tyrosine or serine residues, or involved in binding with specific signalling effectors, such as PI3K, SRC, FAK and AKT. So far, only the crystal structure of the p130CAS SH3 domain has been resolved[134], enabling the search for competitors and specific inhibitors of p130CAS-FAK interaction.

Conclusions

As discussed in this Review, the adaptor proteins of the CAS family, CRK, p140CAP and the IPP complex are crucial mediators of strictly interdependent cellular functions, such as survival, proliferation and migration, which play a key role in transformation and tumour progression. High expression levels of the members of the CAS family, CRK and the IPP complex components mainly drive tumorigenesis by enhancing oncogene signalling, sustaining the ability of cells to grow in anchorage-independent conditions, and to migrate on and invade the ECM. On the contrary, high levels of the p140CAP adaptor negatively regulates tumour phenotype such as cell motility and proliferation, thus possessing tumour suppressive activities.

Since the expression level of these adaptors is crucial for their functions in transformed cells, along with extensive immunohistochemistry studies of human cancers, a detailed screening of gene or chromosome abnormalities in specific cancer types should be undertaken. Moreover, for all of these genes, a deep analysis of the mechanisms that control gene expression, such as promoter structure for transcriptional control, miRNA-based regulation of gene expression and epigenetic modifications, is needed. Identification of exogenous factors such as growth factors, cytokines and chemokines, able to finely tune their relative expression, would also be very important. Interestingly, p130CAS and p140CAP behave as opposing regulators of SRC activity in tumour cells, suggesting the existence of molecular mechanisms that regulate their expression levels, their interaction and the final outcome mediated by the pathway.

As an additional field of investigation, proteomic analysis should clarify the crucial post-translational modifications, such as the presence of phosphorylated residues that can contribute to the activation of specific signalling pathways by recruiting signalling molecules. Silencing of these proteins in different cancer models has been shown to be effective in downregulating or enhancing tumour properties, thus emphasising the importance of these adaptor molecules not only as prognostic markers but also as potential therapeutic targets.

Box1: Integrin signalling and cancer

Integrins are enzymatically inactive receptors, which upon binding to the extracellular matrix (ECM) undergo a conformational change that consequently connects extracellular signals to the intracellular adaptors molecules, such as those discussed in this review, that elicit signal transduction, so-called "outside-in signalling". Integrins have been profoundly implicated in cancer cell proliferation, survival and invasion[1,135,136]. In cancer, integrins are generally aberrantly expressed, rather than present as dominant genetic variants. This aberrant integrin expression can sustain tumours by activating signalling pathways that lead to inhibition of apoptosis, induction of cell proliferation, ECM remodelling, migration and angiogenesis. Integrins are also expressed on tumour-associated cells, such as stromal fibroblasts, endothelial and perivascular cells, bone marrow-derived cells and platelets. The contribution of these cells to cancer progression is highly controlled by integrin signalling[137]. Integrins are also required for metastatic dissemination, including tumour cell migration, invasion and colonization of target tissues[138,139]. Integrin-stimulated pathways are also implicated in the induction of resistance to chemotherapy and ionizing radiation *in vitro*[140].

One mechanism through which integrins are involved in tumour initiation and progression consists of the co-operation between integrins, receptor tyrosine kinases (RTKs) and cytokine receptors. Integrins are required for full tyrosine phosphorylation of the receptors, their binding to signalling molecules and activation of downstream pathways. These signalling cascades generate crucial functional platforms that are important for tumorigenesis, angiogenesis and metastasis [1,2,141,142].

New findings indicate that integrins exert control over the endosomal trafficking of other RTKs, such as epidermal growth factor receptor (EGFR), to regulate cell migration and invasion[143]. Moreover, enhanced integrin-EGFR trafficking is a key mechanism by which the mutant form of tumour suppressor protein p53 can trigger metastasis formation of cancer cells[144]. Therefore, cancer cells are strictly dependent on integrin signalling making the unravelling of the role and functions of integrin signalling effectors of crucial importance to impact on tumour cell biology.

Figure Legends

Figure 1: Main structural features and interactors of integrin adaptors.

A) p130CAS consists of an N-terminal SH3 domain, a substrate domain (SD), a serine rich region (SRR), and a C-terminal domain (CT). The main interactors are indicated. In particular, SRC family kinases (SFKs) bind the CT domain, while the 15 YxxP motifs are phosphorylated by SFK to mediate CRK binding.

B) CRK proteins consist of an N-terminal SH2 domain that binds the phosphorylated SD of p130CAS and of two SH3 domains. The first one interacts with C3G, DOCK180 and ABL1. The second SH3 domain (not present in CRKI) harbours a crucial tyrosine residue (Y221) that is phosphorylated by ARG and ABL1.

C) A schematic representation of the ILK, PINCH, PARVIN (IPP) complex is shown. Integrin-linked kinase (ILK) consists of an N-terminal ankyrin repeat (ANK), a plekstrin homology (PH) and a C-terminal kinase domain. ILK binds to the LIM1 domain of PARVIN and to the second charged amino acid rich domain (CH2) of PINCH. The main interactors of the IPP complex are indicated.

D) p140CAP consists of an N-terminal tyrosine–rich region (Tyr-rich), an actin binding domain (ABD), a proline rich domain (Pro1), a coil-coiled region (C1-C2), two domains rich in charged amino acids (CH1, CH2) and a C-terminal proline rich domain (Pro2). SRC, p130CAS, EB3 and VINEXIN bind to the Pro2 domain of p140CAP. The binding regions of CORTACTIN and CSK have yet to be defined.

Figure 2: **p130CAS and NEDD9 signalling**

(A) Integrins, receptor tyrosine kinases (RTKs) and oestrogen receptor (ER) are major upstream regulators of p130CAS and NEDD9, mainly through the activation of SRC and focal adhesion kinase (FAK) kinases, which phosphorylate p130CAS and NEDD9 and form a CAS-SRC-FAK complex. Tyrosine phosphorylated CAS proteins recruit the adaptor CRK, which in turn binds to several guanine nucleotide exchange factors (GEFS) that switch small GTPases from a GDP-bound inactive to a GTP-bound active state, promoting cell migration. Growth factor receptor-bound protein 2 (GRB2) binding leads to ERK1-ERK2 and PI3K-AKT activation, which sustains cell survival. p130CAS also regulates the invasive program through its interaction with the transcription factor CIZ that acts on metalloproteinases (MMPs) promoter.

(B) In transforming growth factor-β (TGFβ) signalling, p130CAS mediates integrin-dependent suppression of TGFβ–induced apoptosis and growth inhibition. p130CAS binding to SMAD3 reduces TGFβ–dependent SMAD3 phosphorylation leading to growth arrest. p130CAS over-

expression increases SMAD3 coupling to p38 MAPK thus inhibiting TGFβ-induced growth arrest and enhancing the metastatic potential. NEDD9 has been reported as a transcriptional target of TGFβ signalling and enhances cell motility in response to TGFβ.

(C) Upon pro-apoptotic stimuli, CAS proteins undergo dephosphorylation by protein tyrosine phosphatase-PEST (PTP-PEST, also known as PTPN12) and PTPN1 phosphatases resulting in the disassembly of CAS-dependent signalling complexes. Moreover, p130CAS and NEDD9 dephosphorylation favours caspase-dependent cleavage with the production of smaller fragments that bind to E2A and enter the nucleus, contributing to cell death by transcriptional repression of *CDKN1A* (which encodes p21). NEDD9 association with AURORA kinase leads to cell cycle arrest due to inhibition of cytokinesis induced by centrosome amplification and multipolar spindles.

Figure 3. The signalling platform formed by the IPP complex.

The IPP complex is formed by integrin-linked kinase (ILK), PINCH (1 and 2) and PARVIN (α, β and γ). ILK also associates with the cytoplasmic tails of integrin β1. PINCH isoforms bind to receptor tyrosine kinases (RTKs) through the SH2–SH3 adaptor NCK2, thereby coupling growth-factor signalling to integrin signalling. PINCH1 binds to RAS suppressor protein 1 (RSU1) and thymosin-β4 (Tβ4) to influence JUN N-terminal kinase (JNK) signalling and cell migration and survival, respectively. PARVIN α and PARVIN β can bind to F-actin directly, as well as indirectly through binding to paxillin, HIC5 or α-actinin.

Figure 4: p140CAP regulation of intracellular signalling

A) p140CAP binds directly to the SH3 domain of the SRC and CSK kinases. The formation of this molecular complex leads to the activation of CSK, which phosphorylates the inhibitory tyrosine 530 on SRC. The phosphorylation of this site results in a closed inactive SRC conformation.

B) Upon cell matrix adhesion or mitogen stimulus, p140CAP inhibits SRC kinase activity and downstream signalling, inhibiting the SRC-dependent phosphorylation of tyrosine 925 on Focal Adhesion Kinase (FAK) and p130CAS tyrosine phosphorylation. As a consequence, in cells expressing high levels of p140CAP, upon integrin-mediated adhesion, the association between SRC and FAK is impaired. p130CAS phosphorylation leads to the assembly of a p130CAS-CRK signalling complex that drives RAC activation. Consistently, elevated levels of p140CAP severely impair integrin-dependent RAC activity, while its down-regulation induces sustained RAC activation. Moreover, by inactivating SRC, p140CAP also regulates the EGFR pathway through E-CADHERIN-dependent inactivation of EGFR signalling. p140CAP functionally interacts with E-CADHERIN and EGFR at the cell membrane, immobilizes E- CADHERIN at the cell membrane

and increases the interaction between E- CADHERIN and EGFR. As an alternative mechanism, p140CAP also impairs the RAS pathway.

Table I: The expression of integrin adaptors in human tumour malignancies

Integrin adaptor	**Type of malignancy**	**Comments**	**Reference**
p130CAS (*BCAR1*)	Breast cancer	ER positive: Resistance to tamoxifen treatment, high risk of relapse and loss of ER expression ERBB2 positive: Increased proliferation and low prognosis	13,15-17,123
	Chronic myelogenous leukaemia and acute lymphoblastic leukaemia	High levels of expression	145
NEDD9	Melanoma	High level of expression in metastatic melanoma	19
	T-cell leukaemias and virally-induced leukaemias	High levels of expression and hyperphosphorylation	146
	Head and neck squamous cell carcinoma (HNSCC)	High level of expression in metastatic HNSCC	20
CRK	Lung adenocarcinoma, colon cancer and glioblastoma	High levels of expression correlate with tumour aggressiveness, poor prognosis and shorter survival	24-26
p140CAP (*SRCIN1*)	Breast cancer	Decreased protein expression correlates with increased malignancy. mRNA expression positively correlates with unfavourable prognostic factors	39 48
ILK	Colon cancer, melanoma, non-small-cell lung cancer, gastric cancer, pancreatic cancer, prostate cancer	High levels of expression correlates with tumour progression, metastasis and/or poor prognosis	45,147-151
	Colorectal cancer, ovarian Cancer, mesothelioma, Ewing sarcoma, primitive neuroectodermal tumour, medulloblastoma	High levels of expression	152-155
PINCH	Oesophageal cancer, gliomas, oral squamous cell carcinoma, colorectal cancer,	Increased protein expression correlates with tumour aggressiveness	37,38,156-159

	ductal and lobular breast cancer, prostate cancer, lung adenocarcinoma, squamous and basal skin carcinomas, melanoma		
PARVIN-β	Breast cancer	Low expression levels in advanced tumors	[36]

BCAR1, breast cancer anti-estrogen resistance 1; ER, oestrogen receptor; ILK, integrin-linked kinase; *SRCIN1*, SRC kinase signalling inhibitor 1.

Table II: Mouse models of integrin adaptors in cancer.

Adaptor	Mouse model	Phenotype	Refs
p130CAS (*Bcar1*)	MMTV-*Bcar1*	Mammary gland hyperplasia during pregnancy and delayed involution	[13]
	MMTV-*Bcar1*;*NeuT*	Accelerated onset of focal mammary tumours, characterized by upregulation of downstream signalling pathways, leading to cell survival and proliferation	[13]
NEDD9	MMTVPyMT; *Nedd9*$^{-/-}$	Delayed mammary tumour onset, reduced tumour size and number.	[21]
CRK	MMTV-*CRK*	Alteration of mammary epithelium with low incidence of spontaneous mammary epithelial tumours	[28]
ILK	MMTV-*Ilk*	Mammary gland hyperplasia with low frequency of tumour formation	[33]
	MMTV-*Ilk-wnt1*	Acceleration of mammary tumour incidence and growth	[35]
	Ilk$^{fl/fl}$; MMTV-*Erbb2*-IRES-Cre	Delayed tumour growth *in vivo* and inhibition of invasive properties *in vitro*	[34]

Bcar1, breast cancer anti-estrogen resistance 1; ILK, integrin-linked kinase; IRES, internal ribosome entry site; MMTV, mouse mammary tumour virus; PyMT, polyoma middle T.

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References

1. Desgrosellier, J.S. & Cheresh, D.A. Integrins in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer* **10**, 9-22.
2. Cabodi, S. et al. Integrins and signal transduction. *Adv Exp Med Biol* **674**, 43-54.
3. Tikhmyanova, N., Little, J.L. & Golemis, E.A. CAS proteins in normal and pathological cell growth control. *Cell Mol Life Sci* (2009).
4. Defilippi, P., Di Stefano, P. & Cabodi, S. p130Cas: a versatile scaffold in signaling networks. *Trends Cell Biol* **16**, 257-63 (2006).
5. Birge, R.B., Kalodimos, C., Inagaki, F. & Tanaka, S. Crk and CrkL adaptor proteins: networks for physiological and pathological signaling. *Cell Commun Signal* **7**, 13 (2009).
6. Wickstrom, S.A., Lange, A., Montanez, E. & Fassler, R. The ILK/PINCH/parvin complex: the kinase is dead, long live the pseudokinase! *EMBO J* **29**, 281-91 (2009).
7. Singh, M.K. et al. A novel Cas family member, HEPL, regulates FAK and cell spreading. *Mol Biol Cell* **19**, 1627-36 (2008).
8. Ishino, M., Ohba, T., Sasaki, H. & Sasaki, T. Molecular cloning of a cDNA encoding a phosphoprotein, Efs, which contains a Src homology 3 domain and associates with Fyn. *Oncogene* **11**, 2331-8 (1995).
9. Honda, H. et al. Cardiovascular anomaly, impaired actin bundling and resistance to Src-induced transformation in mice lacking p130Cas. *Nat Genet* **19**, 361-5 (1998). **This paper describes the essential role of p130CAS in development and SRC-induced transformation.**
10. Ambrogio, C. et al. p130Cas mediates the transforming properties of the anaplastic lymphoma kinase. *Blood* **106**, 3907-16 (2005).
11. Cabodi, S. et al. p130Cas is an essential transducer element in ErbB2 transformation. *FASEB J.* **10**, 3796-808 (2010). **The preclinical studies reported in this paper highlight the potential use of p130CAS as a novel therapeutic target in ErbB2 positive breast cancers.**
12. Pylayeva, Y. et al. Ras- and PI3K-dependent breast tumorigenesis in mice and humans requires focal adhesion kinase signaling. *J Clin Invest* **119**, 252-66 (2009).
13. Cabodi, S. et al. p130Cas as a new regulator of mammary epithelial cell proliferation, survival, and HER2-neu oncogene-dependent breast tumorigenesis. *Cancer Res* **66**, 4672-80 (2006). **This paper establishes the relevance of p130CAS in ERBB2 tumorigenesis**.
14. Tornillo, G., Bisarò, B., Camacho-Leal, M.P., Galiè, M.,Provero, P., Di Stefano, P., Turco, E., Defilippi, P. and Cabodi, S., . p130Cas promotes invasiveness of three dimensional ErbB2-transformed mammary acinar

structures by enhanced activation of mTOR/p70S6K and Rac1. *EJCB* **in press** (2010).

15. van der Flier, S. et al. BCAR1/p130Cas expression in untreated and acquired tamoxifen-resistant human breast carcinomas. *Int J Cancer* **89**, 465-8 (2000).
16. van der Flier, S. et al. Immunohistochemical study of the BCAR1/p130Cas protein in non-malignant and malignant human breast tissue. *Int J Biol Markers* **16**, 172-8 (2001).
17. Dorssers, L.C. et al. The prognostic value of BCAR1 in patients with primary breast cancer. *Clin Cancer Res* **10**, 6194-202 (2004).
18. Chin, L. Modeling malignant melanoma in mice: pathogenesis and maintenance. *Oncogene* **18**, 5304-10 (1999).
19. Kim, M. et al. Comparative oncogenomics identifies NEDD9 as a melanoma metastasis gene. *Cell* **125**, 1269-81 (2006). **This paper identifies and validates NEDD9 as a highly relevant cancer gene governing metastatic potential in human melanoma.**
20. Lucas, J.T., Jr., Salimath, B.P., Slomiany, M.G. & Rosenzweig, S.A. Regulation of invasive behavior by vascular endothelial growth factor is HEF1-dependent. *Oncogene*.

21. Izumchenko, E. et al. NEDD9 promotes oncogenic signaling in mammary tumor development. *Cancer Res* **69**, 7198-206 (2009). **This study provides the first in vivo evidence of a role for NEDD9 in breast cancer progression.**
22. Matsuda, M. et al. Two species of human CRK cDNA encode proteins with distinct biological activities. *Mol Cell Biol* **12**, 3482-9 (1992).
23. ten Hoeve, J., Morris, C., Heisterkamp, N. & Groffen, J. Isolation and chromosomal localization of CRKL, a human crk-like gene. *Oncogene* **8**, 2469-74 (1993).
24. Miller, C.T. et al. Increased C-CRK proto-oncogene expression is associated with an aggressive phenotype in lung adenocarcinomas. *Oncogene* **22**, 7950-7 (2003).
25. Nishihara, H. et al. Molecular and immunohistochemical analysis of signaling adaptor protein Crk in human cancers. *Cancer Lett* **180**, 55-61 (2002).
26. Takino, T. et al. CrkI adapter protein modulates cell migration and invasion in glioblastoma. *Cancer Res* **63**, 2335-7 (2003).
27. Wang, L. et al. Signaling adaptor protein Crk is indispensable for malignant feature of glioblastoma cell line KMG4. *Biochem Biophys Res Commun* **362**, 976-81 (2007).
28. Fathers, K.E. et al. CrkII transgene induces atypical mammary gland development and tumorigenesis. *Am J Pathol* **176**, 446-60.
29. Eke, I., Hehlgans, S. & Cordes, N. There's something about ILK. *Int J Radiat Biol* **85**, 929-36 (2009).
30. McDonald, P.C., Fielding, A.B. & Dedhar, S. Integrin-linked kinase--essential roles in physiology and cancer biology. *J Cell Sci* **121**, 3121-32 (2008).
31. Hannigan, G., Troussard, A.A. & Dedhar, S. Integrin-linked kinase: a cancer therapeutic target unique among its ILK. *Nat Rev Cancer* **5**, 51-63 (2005).
32. Haase, M. et al. Expression of integrin-linked kinase is increased in differentiated cells. *J Histochem Cytochem* **56**, 819-29 (2008).
33. White, D.E., Cardiff, R.D., Dedhar, S. & Muller, W.J. Mammary epithelial-specific expression of the integrin-linked kinase (ILK) results in the induction of mammary gland hyperplasias and tumors in transgenic mice. *Oncogene* **20**, 7064-72 (2001). **This article provides the first direct demonstration of a potential oncogenic role for ILK.**
34. Pontier, S.M. et al. Integrin-linked kinase has a critical role in ErbB2 mammary tumor progression: implications for human breast cancer. *Oncogene*.
35. Oloumi, A. et al. Cooperative signaling between Wnt1 and integrin-linked kinase induces accelerated breast tumor development. *Breast Cancer Res* **12**, R38.
36. Mongroo, P.S. et al. Beta-parvin inhibits integrin-linked kinase signaling and is downregulated in breast cancer. *Oncogene* **23**, 8959-70 (2004).
37. Wang-Rodriguez, J., Dreilinger, A.D., Alsharabi, G.M. & Rearden, A. The signaling adapter protein PINCH is up-regulated in the stroma of common cancers, notably at invasive edges. *Cancer* **95**, 1387-95 (2002).
38. Gao, J., Arbman, G., Rearden, A. & Sun, X.F. Stromal staining for PINCH is an independent prognostic indicator in colorectal cancer. *Neoplasia* **6**, 796-801 (2004).
39. Damiano, L. et al. p140Cap dual regulation of E-cadherin/EGFR cross-talk and Ras signalling in tumour cell scatter and proliferation. *Oncogene* **29**, 3677-90. **This study shows that p140CAP mechanistically behaves as a tumour suppressor and its expression is lost in more aggressive human breast cancers.**
40. Di Stefano, P. et al. p140Cap protein suppresses tumour cell properties, regulating Csk and Src kinase activity. *Embo J* **26**, 2843-55 (2007).
41. Di Stefano, P. et al. P130Cas-associated protein (p140Cap) as a new tyrosine-phosphorylated protein involved in cell spreading. *Mol Biol Cell* **15**, 787-800 (2004).
42. Chin, L.S., Nugent, R.D., Raynor, M.C., Vavalle, J.P. & Li, L. SNIP, a novel SNAP-25-interacting protein implicated in regulated exocytosis. *J Biol Chem* **275**, 1191-200. (2000).

43. Semba, K. et al. A novel murine gene, Sickle tail, linked to the Danforth's short tail locus, is required for normal development of the intervertebral disc. *Genetics* **172**, 445-56 (2006).
44. Karasugi, T. et al. Association of the tag SNPs in the human SKT gene (KIAA1217) with lumbar disc herniation. *J Bone Miner Res* **24**, 1537-43 (2009).
45. Ito, H. et al. Characterization of a multidomain adaptor protein, p140Cap, as part of a pre-synaptic complex. *J Neurochem* **107**, 61-72 (2008).
46. DeGiorgis, J.A. et al. Phosphoproteomic analysis of synaptosomes from human cerebral cortex. *J Proteome Res* **4**, 306-15 (2005).
47. Collins, M.O. et al. Proteomic analysis of in vivo phosphorylated synaptic proteins. *J Biol Chem* **280**, 5972-82 (2005).
48. Kennedy, S. et al. SNIP/p140Cap mRNA expression is an unfavourable prognostic factor in breast cancer and is not expressed in normal breast tissue. *Br J Cancer* **98**, 1641-5 (2008).
49. Katoh, M. Evolutionary recombination hotspot around GSDML-GSDM locus is closely linked to the oncogenomic recombination hotspot around the PPP1R1B-ERBB2-GRB7 amplicon. *Int J Oncol* **24**, 757-63 (2004).
50. Ruest, P.J., Shin, N.Y., Polte, T.R., Zhang, X. & Hanks, S.K. Mechanisms of CAS substrate domain tyrosine phosphorylation by FAK and Src. *Mol Cell Biol* **21**, 7641-52 (2001).
51. Heasman, S.J. & Ridley, A.J. Mammalian Rho GTPases: new insights into their functions from in vivo studies. *Nat Rev Mol Cell Biol* **9**, 690-701 (2008).
52. Soderling, S.H. Grab your partner with both hands: cytoskeletal remodeling by Arp2/3 signaling. *Sci Signal* **2**, pe5 (2009).
53. Petrie, R.J., Doyle, A.D. & Yamada, K.M. Random versus directionally persistent cell migration. *Nat Rev Mol Cell Biol* **10**, 538-49 (2009).
54. Kobashigawa, Y. et al. Structural basis for the transforming activity of human cancer-related signaling adaptor protein CRK. *Nat Struct Mol Biol* **14**, 503-10 (2007). **This study determines the solution structures of CRK proteins and the molecular mechanism that gives rise to their activities.**
55. Holcomb, M., Rufini, A., Barila, D. & Klemke, R.L. Deregulation of proteasome function induces Abl-mediated cell death by uncoupling p130CAS and c-CrkII. *J Biol Chem* **281**, 2430-40 (2006).
56. Janssen, H. & Marynen, P. Interaction partners for human ZNF384/CIZ/NMP4--zyxin as a mediator for p130CAS signaling? *Exp Cell Res* **312**, 1194-204 (2006).
57. Pratt, S.J. et al. The LIM protein Ajuba influences p130Cas localization and Rac1 activity during cell migration. *J Cell Biol* **168**, 813-24 (2005).
58. Yi, J. et al. Members of the Zyxin family of LIM proteins interact with members of the p130Cas family of signal transducers. *J Biol Chem* **277**, 9580-9 (2002).
59. Nakamoto, T. et al. CIZ, a zinc finger protein that interacts with p130(cas) and activates the expression of matrix metalloproteinases. *Mol Cell Biol* **20**, 1649-58 (2000).
60. Brabek, J. et al. Crk-associated substrate tyrosine phosphorylation sites are critical for invasion and metastasis of SRC-transformed cells. *Mol Cancer Res* **3**, 307-15 (2005).
61. Vultur, A. et al. SKI-606 (bosutinib), a novel Src kinase inhibitor, suppresses migration and invasion of human breast cancer cells. *Mol Cancer Ther* **7**, 1185-94 (2008).
62. Yu, H., Mouw, J.K. & Weaver, V.M. Forcing form and function: biomechanical regulation of tumor evolution. *Trends Cell Biol*. Epub ahead of print
63. Moore, S.W., Roca-Cusachs, P. & Sheetz, M.P. Stretchy proteins on stretchy substrates: the important elements of integrin-mediated rigidity sensing. *Dev Cell* **19**, 194-206.
64. Sawada, Y. et al. Force sensing by mechanical extension of the Src family kinase substrate p130Cas. *Cell* **127**, 1015-26 (2006).
65. Kostic, A. & Sheetz, M.P. Fibronectin rigidity response through Fyn and p130Cas recruitment to the leading edge. *Mol Biol Cell* **17**, 2684-95 (2006).

66. Sanz-Moreno, V. et al. Rac activation and inactivation control plasticity of tumor cell movement. *Cell* **135**, 510-23 (2008).
67. Natarajan, M. et al. HEF1 is a necessary and specific downstream effector of FAK that promotes the migration of glioblastoma cells. *Oncogene* **25**, 1721-32 (2006).
68. Simpson, K.J. et al. Identification of genes that regulate epithelial cell migration using an siRNA screening approach. *Nat Cell Biol* **10**, 1027-38 (2008).
69. Massague, J. TGFbeta in Cancer. *Cell* **134**, 215-30 (2008).
70. Thiery, J.P., Acloque, H., Huang, R.Y. & Nieto, M.A. Epithelial-mesenchymal transitions in development and disease. *Cell* **139**, 871-90 (2009).
71. Zhang, H. et al. Transforming growth factor-beta 1-induced apoptosis is blocked by beta 1-integrin-mediated mitogen-activated protein kinase activation in human hepatoma cells. *Cancer Sci* **95**, 878-86 (2004).
72. Kim, W. et al. The integrin-coupled signaling adaptor p130Cas suppresses Smad3 function in transforming growth factor-beta signaling. *Mol Biol Cell* **19**, 2135-46 (2008).
73. Wendt, M.K., Smith, J.A. & Schiemann, W.P. p130Cas is required for mammary tumor growth and transforming growth factor-beta-mediated metastasis through regulation of Smad2/3 activity. *J Biol Chem* **284**, 34145-56 (2009).
74. Giampieri, S. et al. Localized and reversible TGFbeta signalling switches breast cancer cells from cohesive to single cell motility. *Nat Cell Biol* **11**, 1287-96 (2009).
75. Thiery, J.P. & Sleeman, J.P. Complex networks orchestrate epithelial-mesenchymal transitions. *Nat Rev Mol Cell Biol* **7**, 131-42 (2006).
76. Shintani, Y., Hollingsworth, M.A., Wheelock, M.J. & Johnson, K.R. Collagen I promotes metastasis in pancreatic cancer by activating c-Jun NH(2)-terminal kinase 1 and up-regulating N-cadherin expression. *Cancer Res* **66**, 11745-53 (2006).
77. Shintani, Y., Wheelock, M.J. & Johnson, K.R. Phosphoinositide-3 kinase-Rac1-c-Jun NH2-terminal kinase signaling mediates collagen I-induced cell scattering and up-regulation of N-cadherin expression in mouse mammary epithelial cells. *Mol Biol Cell* **17**, 2963-75 (2006).
78. Shintani, Y. et al. Collagen I-mediated up-regulation of N-cadherin requires cooperative signals from integrins and discoidin domain receptor 1. *J Cell Biol* **180**, 1277-89 (2008).
79. Bhattacharya, S., Guo, H., Ray, R.M. & Johnson, L.R. Basic helix-loop-helix protein E47-mediated p21Waf1/Cip1 gene expression regulates apoptosis of intestinal epithelial cells. *Biochem J* **407**, 243-54 (2007).
80. Kim, W., Kook, S., Kim, D.J., Teodorof, C. & Song, W.K. The 31-kDa caspase-generated cleavage product of p130cas functions as a transcriptional repressor of E2A in apoptotic cells. *J Biol Chem* **279**, 8333-42 (2004).
81. Law, S.F., O'Neill, G.M., Fashena, S.J., Einarson, M.B. & Golemis, E.A. The docking protein HEF1 is an apoptotic mediator at focal adhesion sites. *Mol Cell Biol* **20**, 5184-95 (2000).
82. Daouti, S. et al. A selective phosphatase of regenerating liver phosphatase inhibitor suppresses tumor cell anchorage-independent growth by a novel mechanism involving p130Cas cleavage. *Cancer Res* **68**, 1162-9 (2008).
83. Achiwa, H. & Lazo, J.S. PRL-1 tyrosine phosphatase regulates c-Src levels, adherence, and invasion in human lung cancer cells. *Cancer Res* **67**, 643-50 (2007).
84. Wendt, M.K., Drury, L.J., Vongsa, R.A. & Dwinell, M.B. Constitutive CXCL12 expression induces anoikis in colorectal carcinoma cells. *Gastroenterology* **135**, 508-17 (2008).
85. Gasparini, G., Longo, R., Sarmiento, R. & Morabito, A. Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents? *Lancet Oncol* **4**, 605-15 (2003).
86. Casanova, I. et al. Celecoxib induces anoikis in human colon carcinoma cells associated with the deregulation of focal adhesions and nuclear translocation of p130Cas. *Int J Cancer* **118**, 2381-9 (2006).

87. Casanova, I. et al. A celecoxib derivative inhibits focal adhesion signaling and induces caspase-8-dependent apoptosis in human acute myeloid leukemia cells. *Int J Cancer* **123**, 217-26 (2008).
88. Sakai, R., Nakamoto, T., Ozawa, K., Aizawa, S. & Hirai, H. Characterization of the kinase activity essential for tyrosine phosphorylation of p130Cas in fibroblasts. *Oncogene* **14**, 1419-26 (1997).
89. Iwahara, T., Akagi, T., Fujitsuka, Y. & Hanafusa, H. CrkII regulates focal adhesion kinase activation by making a complex with Crk-associated substrate, p130Cas. *Proc Natl Acad Sci U S A* **101**, 17693-8 (2004).
90. Akagi, T., Shishido, T., Murata, K. & Hanafusa, H. v-Crk activates the phosphoinositide 3-kinase/AKT pathway in transformation. *Proc Natl Acad Sci U S A* **97**, 7290-5 (2000).
91. Watanabe, T. et al. Crk adaptor protein-induced phosphorylation of Gab1 on tyrosine 307 via Src is important for organization of focal adhesions and enhanced cell migration. *Cell Res* **19**, 638-50 (2009).
92. Watanabe, T. et al. Adaptor molecule Crk is required for sustained phosphorylation of Grb2-associated binder 1 and hepatocyte growth factor-induced cell motility of human synovial sarcoma cell lines. *Mol Cancer Res* **4**, 499-510 (2006).
93. Crawford, M. et al. MicroRNA-126 inhibits invasion in non-small cell lung carcinoma cell lines. *Biochem Biophys Res Commun* **373**, 607-12 (2008).
94. Legate, K.R., Montanez, E., Kudlacek, O. & Fassler, R. ILK, PINCH and parvin: the tIPP of integrin signalling. *Nat Rev Mol Cell Biol* **7**, 20-31 (2006).
95. Attwell, S., Mills, J., Troussard, A., Wu, C. & Dedhar, S. Integration of cell attachment, cytoskeletal localization, and signaling by integrin-linked kinase (ILK), CH-ILKBP, and the tumor suppressor PTEN. *Mol Biol Cell* **14**, 4813-25 (2003).
96. Yamaji, S. et al. A novel integrin-linked kinase-binding protein, affixin, is involved in the early stage of cell-substrate interaction. *J Cell Biol* **153**, 1251-64 (2001).
97. Lange, A. et al. Integrin-linked kinase is an adaptor with essential functions during mouse development. *Nature* **461**, 1002-6 (2009).
98. Maydan, M. et al. Integrin-linked kinase is a functional Mn2+-dependent protein kinase that regulates glycogen synthase kinase-3beta (GSK-3beta) phosphorylation. *PLoS One* **5**, e12356.
99. Fukuda, K., Gupta, S., Chen, K., Wu, C. & Qin, J. The pseudoactive site of ILK is essential for its binding to alpha-Parvin and localization to focal adhesions. *Mol Cell* **36**, 819-30 (2009).
100. Somasiri, A., Howarth, A., Goswami, D., Dedhar, S. & Roskelley, C.D. Overexpression of the integrin-linked kinase mesenchymally transforms mammary epithelial cells. *J Cell Sci* **114**, 1125-36 (2001).
101. Filipenko, N.R., Attwell, S., Roskelley, C. & Dedhar, S. Integrin-linked kinase activity regulates Rac- and Cdc42-mediated actin cytoskeleton reorganization via alpha-PIX. *Oncogene* **24**, 5837-49 (2005).
102. Troussard, A.A. et al. The integrin linked kinase (ILK) induces an invasive phenotype via AP-1 transcription factor-dependent upregulation of matrix metalloproteinase 9 (MMP-9). *Oncogene* **19**, 5444-52 (2000).
103. Fielding, A.B., Dobreva, I., McDonald, P.C., Foster, L.J. & Dedhar, S. Integrin-linked kinase localizes to the centrosome and regulates mitotic spindle organization. *J Cell Biol* **180**, 681-9 (2008). **These data demonstrate an unexpected function for ILK in the organization of centrosomal protein complexes during mitotic spindle assembly and DNA segregation.**
104. Fielding, A.B., Lim, S., Montgomery, K., Dobreva, I. & Dedhar, S. A critical role of integrin-linked kinase, ch-TOG and TACC3 in centrosome clustering in cancer cells. *Oncogene*. Epub ahead of print (2010).

105. Durbin, A.D., Hannigan, G.E. & Malkin, D. Oncogenic ILK, tumor suppression and all that JNK. *Cell Cycle* **8**, 4060-6 (2009). **This work provides a mechanism for interconversion of oncogenic and tumour-suppressor functions of ILK in rhabdomyosarcoma.**
106. Troussard, A.A. et al. Preferential dependence of breast cancer cells versus normal cells on integrin-linked kinase for protein kinase B/Akt activation and cell survival. *Cancer Res* **66**, 393-403 (2006).
107. Durbin, A.D. et al. JNK1 determines the oncogenic or tumor-suppressive activity of the integrin-linked kinase in human rhabdomyosarcoma. *J Clin Invest* **119**, 1558-70 (2009).
108. Wexler, L.H., Crist, W.M., and Helman, L.J. . in In Principles and practice of pediatric oncology (ed. P.A. Pizzo and D.G. Poplack) 939-971. (Lippincott Williams & Wilkins. , Philadelphia, Pennsylvania, USA., 2002).
109. Fukuda, T., Chen, K., Shi, X. & Wu, C. PINCH-1 is an obligate partner of integrin-linked kinase (ILK) functioning in cell shape modulation, motility, and survival. *J Biol Chem* **278**, 51324-33 (2003).
110. Johnstone, C.N. et al. Parvin-beta inhibits breast cancer tumorigenicity and promotes CDK9-mediated peroxisome proliferator-activated receptor gamma 1 phosphorylation. *Mol Cell Biol* **28**, 687-704 (2008).
111. Castellvi-Bel, S. et al. Evaluation of PARVG located on 22q13 as a candidate tumor suppressor gene for colorectal and breast cancer. *Cancer Genet Cytogenet* **144**, 80-2 (2003).
112. Chen, K. et al. PINCH-1 regulates the ERK-Bim pathway and contributes to apoptosis resistance in cancer cells. *J Biol Chem* **283**, 2508-17 (2008).
113. Liang, X. et al. Pinch1 is required for normal development of cranial and cardiac neural crest-derived structures. *Circ Res* **100**, 527-35 (2007).
114. Latour, S. & Veillette, A. Proximal protein tyrosine kinases in immunoreceptor signaling. *Curr Opin Immunol* **13**, 299-306 (2001).
115. Perrais, M., Chen, X., Perez-Moreno, M. & Gumbiner, B.M. E-cadherin homophilic ligation inhibits cell growth and epidermal growth factor receptor signaling independently of other cell interactions. *Mol Biol Cell* **18**, 2013-25 (2007).
116. Qian, X., Karpova, T., Sheppard, A.M., McNally, J. & Lowy, D.R. E-cadherin-mediated adhesion inhibits ligand-dependent activation of diverse receptor tyrosine kinases. *Embo J* **23**, 1739-48 (2004).
117. Takahashi, K. & Suzuki, K. Density-dependent inhibition of growth involves prevention of EGF receptor activation by E-cadherin-mediated cell-cell adhesion. *Exp Cell Res* **226**, 214-22 (1996).
118. Prescott, J. et al. Androgen receptor-mediated repression of novel target genes. *Prostate* **67**, 1371-83 (2007).
119. Jaworski, J. et al. Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity. *Neuron* **61**, 85-100 (2009).
120. Cabodi, S. et al. p130Cas interacts with estrogen receptor alpha and modulates non-genomic estrogen signaling in breast cancer cells. *J Cell Sci* **117**, 1603-11 (2004).
121. Brinkman, A., van der Flier, S., Kok, E.M. & Dorssers, L.C. BCAR1, a human homologue of the adapter protein p130Cas, and antiestrogen resistance in breast cancer cells. *J Natl Cancer Inst* **92**, 112-20 (2000). **This paper points out the correlation of p130CAS expression with anti-oestrogen resistance in breast cancer.**
122. van Agthoven, T. et al. Identification of BCAR3 by a random search for genes involved in antiestrogen resistance of human breast cancer cells. *EMBO J* **17**, 2799-808 (1998).
123. Dorssers, L.C. et al. Tamoxifen resistance in breast cancer: elucidating mechanisms. *Drugs* **61**, 1721-33 (2001).
124. Gotoh, T., Cai, D., Tian, X., Feig, L.A. & Lerner, A. p130Cas regulates the activity of AND-34, a novel Ral, Rap1, and R-Ras guanine nucleotide exchange factor. *J Biol Chem* **275**, 30118-23 (2000).

125. Makkinje, A. et al. AND-34/BCAR3 regulates adhesion-dependent p130Cas serine phosphorylation and breast cancer cell growth pattern. *Cell Signal* **21**, 1423-35 (2009).
126. Brinkman, A. et al. The substrate domain of BCAR1 is essential for anti-estrogen-resistant proliferation of human breast cancer cells. *Breast Cancer Res Treat* (2009).
127. Ta, H.Q., Thomas, K.S., Schrecengost, R.S. & Bouton, A.H. A novel association between p130Cas and resistance to the chemotherapeutic drug adriamycin in human breast cancer cells. *Cancer Res* **68**, 8796-804 (2008).
128. Thao le, B. et al. Cas-L was overexpressed in imatinib-resistant gastrointestinal stromal tumor cells. *Cancer Biol Ther* **8**, 683-8 (2009).
129. Edwards, L.A. et al. Inhibition of ILK in PTEN-mutant human glioblastomas inhibits PKB/Akt activation, induces apoptosis, and delays tumor growth. *Oncogene* **24**, 3596-605 (2005).
130. Wong, R.P., Ng, P., Dedhar, S. & Li, G. The role of integrin-linked kinase in melanoma cell migration, invasion, and tumor growth. *Mol Cancer Ther* **6**, 1692-700 (2007).
131. Edwards, L.A. et al. Suppression of VEGF secretion and changes in glioblastoma multiforme microenvironment by inhibition of integrin-linked kinase (ILK). *Mol Cancer Ther* **7**, 59-70 (2008).
132. Liu, J. et al. Integrin-linked kinase inhibitor KP-392 demonstrates clinical benefits in an orthotopic human non-small cell lung cancer model. *J Thorac Oncol* **1**, 771-9 (2006).
133. Yau, C.Y., Wheeler, J.J., Sutton, K.L. & Hedley, D.W. Inhibition of integrin-linked kinase by a selective small molecule inhibitor, QLT0254, inhibits the PI3K/PKB/mTOR, Stat3, and FKHR pathways and tumor growth, and enhances gemcitabine-induced apoptosis in human orthotopic primary pancreatic cancer xenografts. *Cancer Res* **65**, 1497-504 (2005).
134. Wisniewska, M. et al. The 1.1 A resolution crystal structure of the p130cas SH3 domain and ramifications for ligand selectivity. *J Mol Biol* **347**, 1005-14 (2005). **This study reports the first crystal structure of p130CAS SH3 domain, enabling modelling of the docking interactions, and supporting structure-based drug design of inhibitors of the CAS-FAK interaction.**
135. Assoian, R.K. & Klein, E.A. Growth control by intracellular tension and extracellular stiffness. *Trends Cell Biol* **18**, 347-52 (2008).
136. Streuli, C.H. & Akhtar, N. Signal co-operation between integrins and other receptor systems. *Biochem J* **418**, 491-506 (2009).
137. Desgrosellier, J.S. et al. An integrin alpha(v)beta(3)-c-Src oncogenic unit promotes anchorage-independence and tumor progression. *Nat Med* **15**, 1163-9 (2009). **This paper describes a role for unligated integrin αvβ3 in increasing anchorage-independent survival and metastasis through an integrin αvβ3–SRC signalling module.**
138. Brakebusch, C. & Fassler, R. beta 1 integrin function in vivo: adhesion, migration and more. *Cancer Metastasis Rev* **24**, 403-11 (2005).
139. Christofori, G. New signals from the invasive front. *Nature* **441**, 444-50 (2006).
140. Nam, J.M., Chung, Y., Hsu, H.C. & Park, C.C. beta1 integrin targeting to enhance radiation therapy. *Int J Radiat Biol* **85**, 923-8 (2009).
141. Defilippi, P. et al. {beta}1 Integrin and IL-3R coordinately regulate STAT5 activation and anchorage-dependent proliferation. *J Cell Biol* **168**, 1099-108 (2005).
142. Uberti, B., Dentelli, P., Rosso, A., Defilippi, P.,& Brizzi, M.F. Inhibition of BETA 1 Integrin and IL-3 beta common subunit interaction hinders tumor angiogenesis. *Oncogene* Epub ahead of print (2010).
143. Caswell, P.T., Vadrevu, S. & Norman, J.C. Integrins: masters and slaves of endocytic transport. *Nat Rev Mol Cell Biol* **10**, 843-53 (2009).
144. Muller, P.A. et al. Mutant p53 drives invasion by promoting integrin recycling. *Cell* **139**, 1327-41 (2009).

145. Salgia, R. et al. p130CAS forms a signaling complex with the adapter protein CRKL in hematopoietic cells transformed by the BCR/ABL oncogene. *J Biol Chem* **271**, 25198-203 (1996).
146. Iwata, S. et al. HTLV-I Tax induces and associates with Crk-associated substrate lymphocyte type (Cas-L). *Oncogene* **24**, 1262-71 (2005).
147. Bravou, V., Klironomos, G., Papadaki, E., Taraviras, S. & Varakis, J. ILK over-expression in human colon cancer progression correlates with activation of beta-catenin, down-regulation of E-cadherin and activation of the Akt-FKHR pathway. *J Pathol* **208**, 91-9 (2006).
148. Dai, D.L. et al. Increased expression of integrin-linked kinase is correlated with melanoma progression and poor patient survival. *Clin Cancer Res* **9**, 4409-14 (2003).
149. Takanami, I. Increased expression of integrin-linked kinase is associated with shorter survival in non-small cell lung cancer. *BMC Cancer* **5**, 1 (2005).
150. Sawai, H. et al. Integrin-linked kinase activity is associated with interleukin-1 alpha-induced progressive behavior of pancreatic cancer and poor patient survival. *Oncogene* **25**, 3237-46 (2006).
151. Graff, J.R. et al. Integrin-linked kinase expression increases with prostate tumor grade. *Clin Cancer Res* **7**, 1987-91 (2001).
152. Huang, H.C. et al. Thymosin beta4 triggers an epithelial-mesenchymal transition in colorectal carcinoma by upregulating integrin-linked kinase. *Oncogene* **26**, 2781-90 (2007).
153. Ahmed, N. et al. Integrin-linked kinase expression increases with ovarian tumour grade and is sustained by peritoneal tumour fluid. *J Pathol* **201**, 229-37 (2003).
154. Watzka, S.B. et al. Reactivity of integrin-linked kinase in human mesothelial cell proliferation. *Interact Cardiovasc Thorac Surg* **7**, 107-10 (2008).
155. Chung, D.H. et al. ILK (beta1-integrin-linked protein kinase): a novel immunohistochemical marker for Ewing's sarcoma and primitive neuroectodermal tumour. *Virchows Arch* **433**, 113-7 (1998).
156. Zhu, Z. et al. PINCH expression and its significance in esophageal squamous cell carcinoma. *Dis Markers* **25**, 75-80 (2008).
157. Wang, M.W. et al. Expression of PINCH protein in gliomas and its clinicopathological significance. *Oncology* **72**, 343-6 (2007).
158. Zhang, J.T. et al. Up-regulation of PINCH in the stroma of oral squamous cell carcinoma predicts nodal metastasis. *Oncol Rep* **14**, 1519-22 (2005).
159. Zhao, Z.R. et al. Particularly interesting new cysteine-histidine rich protein expression in colorectal adenocarcinomas. *World J Gastroenterol* **12**, 298-301 (2006).

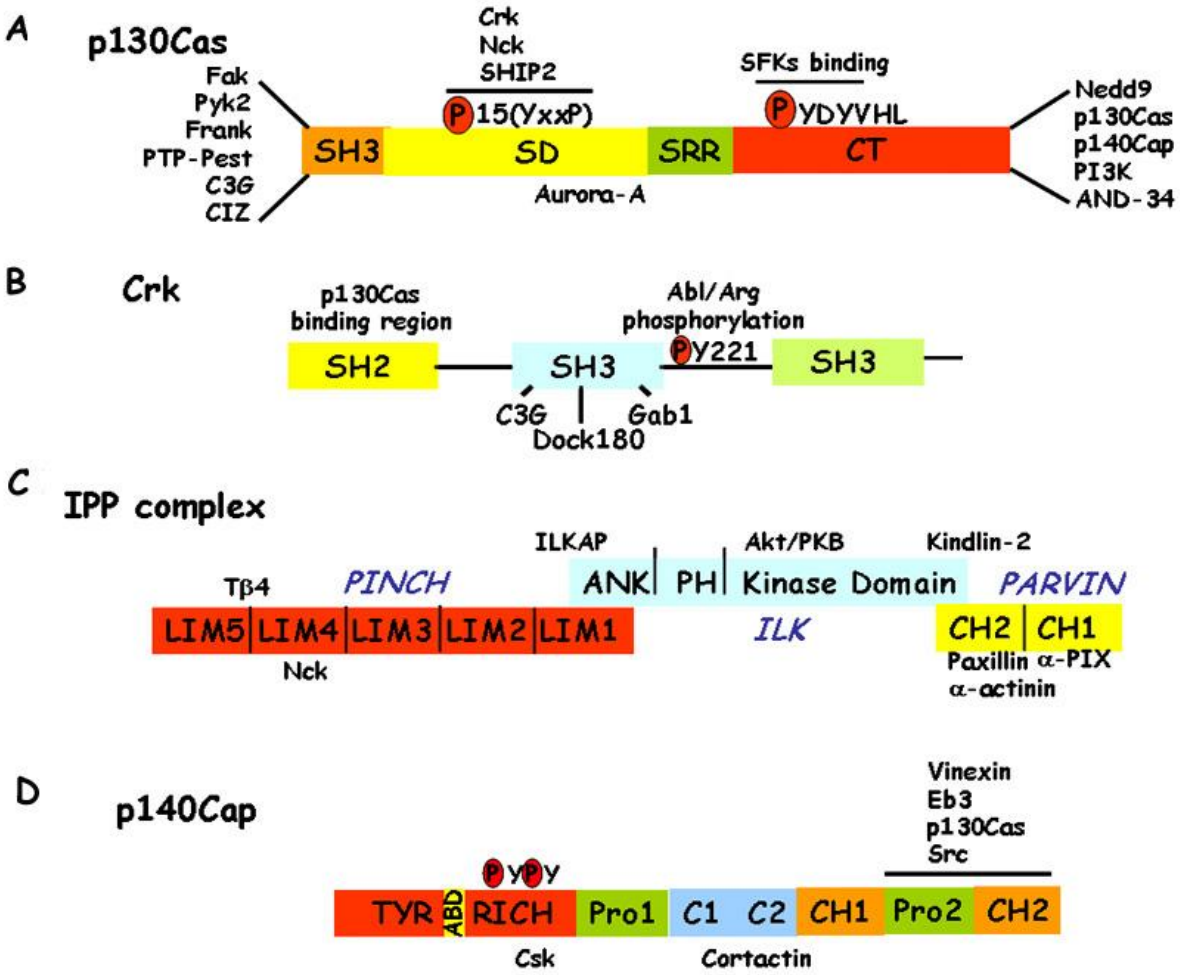
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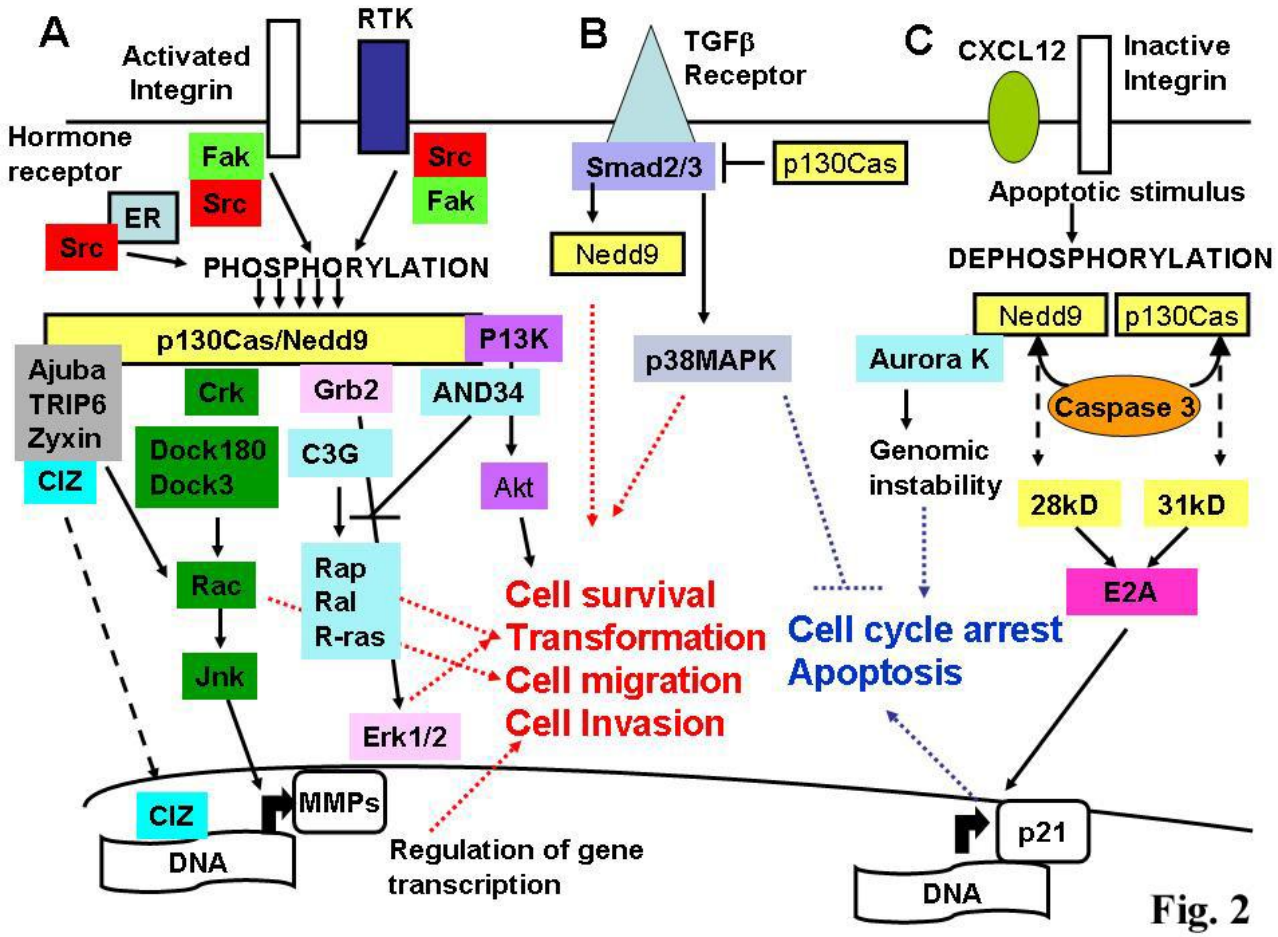
Fig. 1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TRIP6
Zyxin
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Grb2
AND34
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Dock180
Dock3
C3G
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Ral
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Jnk
Erk1/2
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Cell migration
Cell Invasion
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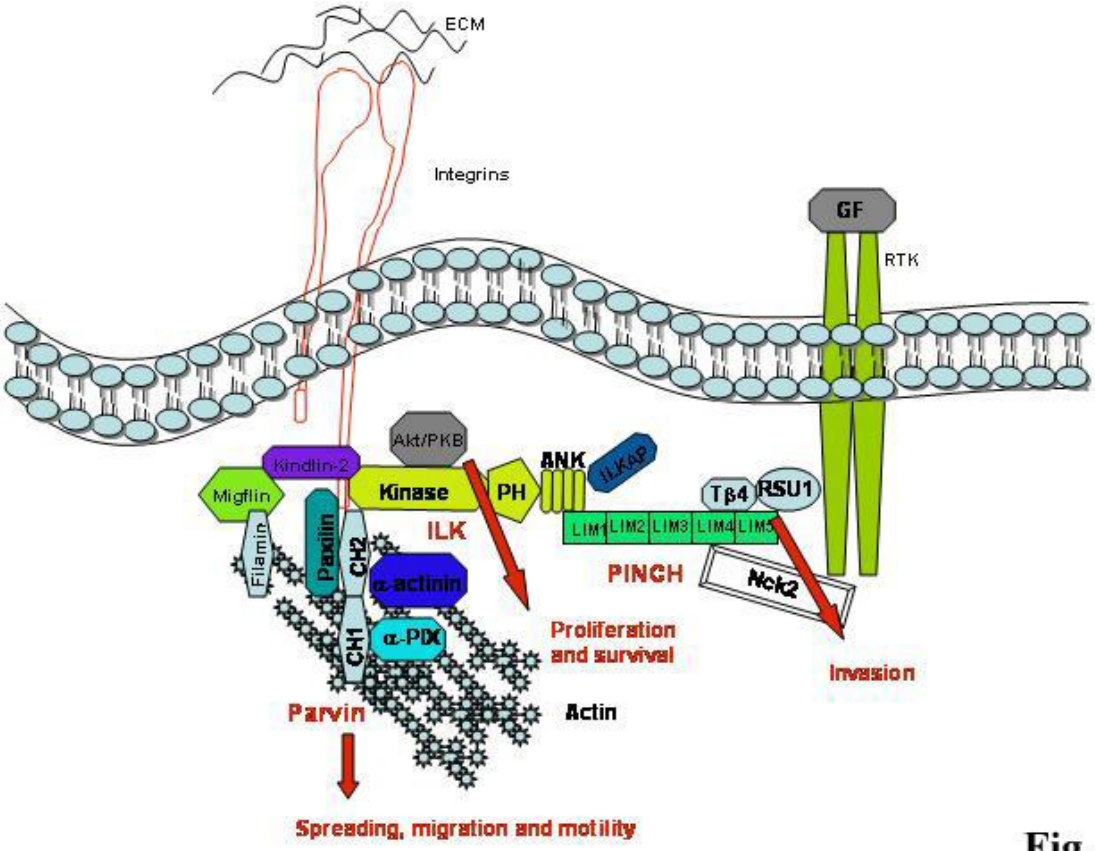
Fig. 2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LIM2
LIM3
LIM4
LIM5
PINCH
Nck2
Filamin
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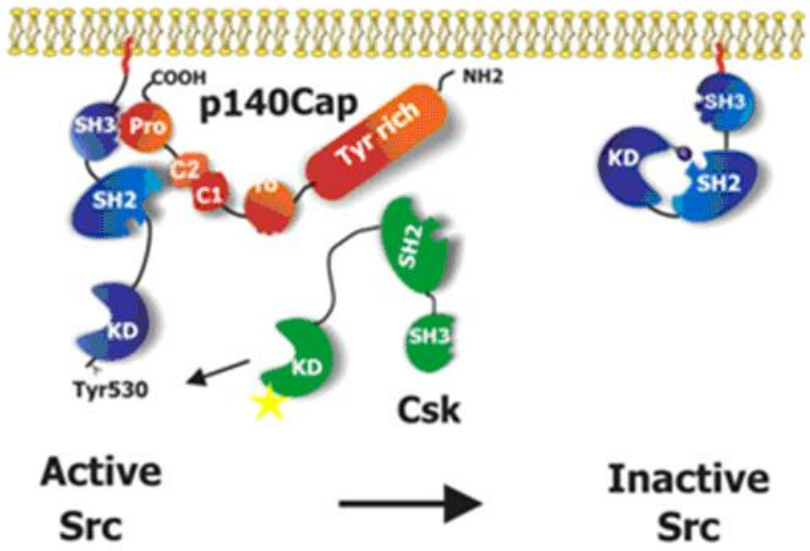
and survival
Invasion
Parvin
Actin
Spreading, migration and motility

Fig. 3

A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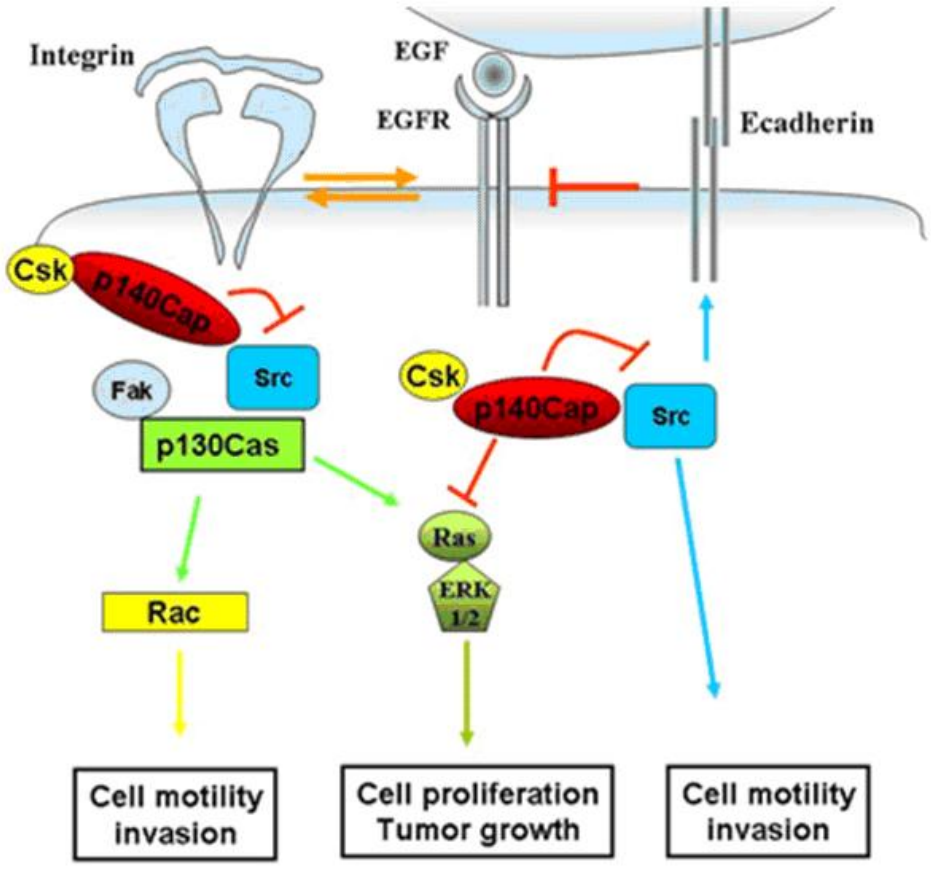
B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Csk
p140Cap
Src
Ras
ERK
1/2
Rac
Cell motility
invasion
Cell proliferation
Tumor growth
Cell motility
invasion

Fig.4

Glossary

G3 index: In the classification of human breast cancer, grade 3 (G3) indicates that the cancer has spread to lymph nodes, regardless of its size.

Podosomes: A type of ECM contact that is different from focal complexes and focal adhesions. They are built around an actin filament core, surrounded by a ring structure of integrin adhesive complexes.

Mesenchymal movement: Movement of cells with elongated morphology and a front-back polarity, with traction generated through integrin-dependent adhesion. This type of motility requires extracellular proteolysis for cell invasion and is thought to depend on RAC1.

Amoeboid motility: This movement is characterized by high speeds, lack of stable polarity and a relatively amorphous cell shape and is frequently exhibited by cancer cells. It does not require stable integrin-dependent adhesion for traction but depends on RHOA to increase actomyosin contractility and allow invasion in the absence of extracellular proteolysis.

Collective motility: Migration of cells as a cohesive group as a hallmark of tissue remodelling during wound repair and cancer invasion. It is characterised by cells moving as sheets, strands, clusters or ducts rather than individually.

Actin stress fibres: They are a self-assembling, structural component of the cytoskeleton, that typically appear as long thick actin bundles that span across the cell body and lie along the ventral surface. By binding to myosin, they produce traction forces and resting tension.

Cortical actin: A concentrated layer of actin filaments that lie longitudinally and roughly parallel to each other just beneath the plasma membrane.

BIOGRAPHY OF THE AUTHORS

Paola Defilippi. Full Professor in Cell Biology, University of Torino. PhD in Biochemistry, Universitè Libre de Bruxelles. The research interest is focused on the identification of integrin and growth factor receptors dependent signalling controlling proliferation and migration of normal and transformed cell.

Sara Cabodi, Assistant Professor in Cell Biology, University of Torino. PhD in Cell Biology, Harvard Medical School/MGH, Boston. The main research interest is the study of the adaptor protein p130CAS in ErbB2 dependent breast tumourigenesis. Preclinical studies to assess the feasibility of using p130CAS for breast cancer therapy.

Maria del Pilar Camacho-Leal, Post-Doc, University of Torino. PhD in Biochemistry, McGill University, Montreal. The main focus of the research is the study of the integrin adaptor molecules p130CAS and p140CAP in colon cancer and the study of the role of p130CAS in oestrogen-positive breast cancer.

Paola Di Stefano, Post-Doc University of Torino. PhD in Cell Biology, University of Torino. The research activity led to the identification of p140CAP and the current research is focused on the study of the involvement of this integrin adaptor protein in normal and cancer cells.

At-a-glance summary

In cancer cells, integrin and growth factor receptor cross-talk leads to the recruitment of integrins signalling adaptors to assemble intracellular signalling platforms that result in cellular transformation and control of migration and invasion. The biological effects regulated by integrin adaptors are dependent on their expression levels and on their phosphorylation status, which determine the association with binding effectors.

p130CAS, NEDD9, CRK, the IPP complex (ILK, PINCH and PARVIN), and p140CAP integrin adaptors have a profound influence on all aspects of cancer progression, including initiation, progression and metastasis. Transgenic and xenograft animal models support the crucial role of these integrin adaptors in tumorigenesis.

In several human tumours, high expression of p130CAS, NEDD9, CRK, ILK and PINCH correlates with increased disease progression, while the levels of PARVIN β and p140CAP proteins are inversely correlated with malignancy. Current knowledge also implicates integrin adaptors in acquired resistance to cancer treatment.

In cancer cells, at the molecular level, these adaptors regulate signalling pathways required for the control of cell proliferation, survival and for actin cytoskeleton organisation and extracellular matrix degradation. These events are fundamental for transformation and cancer progression, highlighting the integrin adaptors as key players in the onset of tumorigenesis.

Targeting integrin adaptors by modulating their expression levels or their activity in different types of cancer, has been proven to be effective for interfering with malignancy, making the integrin adaptors suitable targets for cancer therapy.