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Review

Signalling via integrins: Implications for cell survival and anticancer strategies

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Abstract

Integrin-associated signalling renders cells more resistant to genotoxic anti-cancer agents like ionizing radiation and chemotherapeutic substances, a phenomenon termed cell adhesion-mediated radioresistance/drug resistance (CAM-RR, CAM-DR). Integrins are heterodimeric cell-surface molecules that on one side link the actin cytoskeleton to the cell membrane and on the other side mediate cell–matrix interactions. In addition to their structural functions, integrins mediate signalling from the extracellular space into the cell through integrin-associated signalling and adaptor molecules such as FAK (focal adhesion kinase), ILK (integrin-linked kinase), PINCH (particularly interesting new cysteine-histidine rich protein) and Nck2 (non-catalytic (region of) tyrosine kinase adaptor protein 2). Via these molecules, integrin signalling tightly and cooperatively interacts with receptor tyrosine kinase signalling to regulate survival, proliferation and cell shape as well as polarity, adhesion, migration and differentiation. In tumour cells of diverse origin like breast, colon or skin, the function and regulation of these molecules is partly disturbed and thus might contribute to the malignant phenotype and pre-existent and acquired multidrug resistance. These issues as well as a variety of therapeutic options envisioned to influence tumour cell growth, metastasis and resistance, including kinase inhibitors, anti-integrin antibodies or RNA interference, will be summarized and discussed in this review.

Keywords: ECM; FAK; ILK; Integrin; Nck2; PINCH

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Abbreviations: AKT, protein kinase B/AKT; CAM-DR, cell adhesion-mediated drug resistance; CAM-RR, cell adhesion-mediated radioresistance; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; ERK1/2, extracellular signal-regulated kinase-1/2; FAK, focal adhesion kinase; GSK3β, glycogen synthase kinase-3β; ILK, integrin-linked kinase; ILKAP, ILK-associated protein serine/threonine phosphatase of the PP2C family; JNK, Jun N-terminal kinase; lrBM, laminin-rich basement membrane; MAPK, mitogen-activated protein kinase; MAP4K, mitogen-activated protein kinase kinase kinase kinase; MMP, matrix metalloproteinase; Nck, Non-catalytic (region of) tyrosine kinase adaptor protein; p130Cas, Crk-associated substrate; PAK1, $p21^{rac}$ activated protein kinase; PDGFR, platelet-derived growth factor receptor; PH, pleckstrin homology; PINCH, particularly interesting new cysteine-histidine rich protein; PI3K, phosphatidylinositol-3-kinase; PIP3, phosphatidylinositol 3,4,5-triphosphate; ROCK, Rho-associated coiled-coil-containing kinase; RTK, receptor tyrosine kinase; SH, Src homology; Shc, Src homology containing; TNFα, tumour necrosis factor alpha; VEGF, vascular endothelial growth factor

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1. Introduction

Cell survival depends on multiple signalling inputs like growth factors, nutrients, and attachment to surrounding cells and extracellular matrix (ECM) components. This holds also true for tumours, which often grow seemingly independent of such signals [1–4]. Tumour cells have developed effective mechanisms to replace external input signals by autonomous activation of intracellular pathways, thus, escaping normal growth control and contact inhibition. Successful therapies of tumours have to take into account that multiple survival pathways are, at least partially, able to replace each other. Therefore, tumour therapies that are targeted to crossroads of major survival pathways are likely to be most successful. One such point of convergence is located at distinct cell membrane areas termed focal adhesions (FA), which facilitate cell–ECM interactions [5]. These large multiprotein complexes consist of integrins, integrin-associated adaptor and signalling proteins, growth factor receptors and their related downstream targets. Mutual and cooperative communication between all these molecules forms a complex platform that tightly controls critical cell functions such as cell survival, proliferation, differentiation, adhesion, migration and resistance to chemo- and radiotherapy.

Recent therapeutic approaches targeted diverse growth factor receptors with the members of the EGFR (epidermal growth factor receptor) family as prime example [6]. Although EGFR-targeting humanized monoclonal antibodies and small molecule tyrosine kinase inhibitors effectively reduced cell proliferation in vitro and in vivo, the clinical trials indicated an overall therapeutic response rate of about 10% in monotherapy of non-small cell lung cancer, advanced squamous cell carcinoma of the head and neck, colorectal carcinoma and renal cell carcinoma [6]. Hence, effective blocking of the EGFR pathway seems to be counteracted by yet unknown mechanisms. In addition to the ‘classical’ multidrug resistance phenotype associated with overexpression of e.g. the p-glycoprotein [7], integrin-mediated cell adhesion to ECM and integrin downstream molecules may be considered as potent antagonists of growth factor receptor-targeting therapeutics.

Clinical trials administrating low-molecular-weight integrin inhibitors such as RGD peptides or anti-integrin blocking antibodies are under way (Table 1). First data demonstrated good tolerability and low toxicity in patients, which prompted the initiation of clinical studies using a combination with chemo- or radiotherapy [8].

In contrast to effective but generally toxic chemotherapy, radiotherapy has been developed to a highly sophisticated and successful method to kill tumour cells in circumscribed regions of the human body. On the basis of improved planning and delivery technology, the radiation doses applied are fatal for tumour cells while the surrounding normal tissue is spared [9].

During the last decades the effects of ionizing radiation have been extensively investigated with regard to DNA damage, DNA damage repair, cell cycling, mitotic catastrophe and apoptosis. Studies analyzing radiation-dependent modification of the cellular microenvironment added new facets to our understanding of the cellular behaviour upon irradiation [10,11]. Particularly, potent candidates involved in the regulation of angiogenesis, hypoxia and cell–matrix interactions were identified envisioning possible targeted therapies to increase the therapeutic window of radiotherapy. Considering the effects of ionizing radiation on tissue, the multiple interactions between different cell types, growth factors, cytokines and the ECM come to light. Upon radiation, the tumour–stroma interrelation and ECM remodelling are induced through the activation of proteases, soluble cytokines and growth factors. This activation results from interactions of energy with water creating reactive oxygen species (ROS) as indirect radiation effects. Consequently, the behaviour and the phenotype of each cell in the parenchyma, stroma or tumour are modulated likely contributing to the development of acquired cellular resistance against genotoxic anticancer agents; an observation clearly shown for integrin-mediated tumour cell adhesion to ECM.

The following review will focus on integrins and major integrin-associated proteins such as FAK, ILK, PINCH and Nck2, which are mediators of survival, motility and attachment pathways frequently out of control in tumour cells [12–15]. The emphasis is placed on a distinct, in-depth explored subset of molecules and their role in the response to conventional tumour therapies, like chemotherapy and irradiation, and their potential impact in targeted tumour therapies.

2. Integrin signalling

Cell adhesion molecules of the integrin family consist of 18 α and 8 β subunits which form 24 known αβ-heterodimers depending on cell type and cellular function (reviewed in [13,16,17]). Each integrin subunit has a large extracellular, a short transmembrane and small intracellular domain with a total of >1600 amino acids. Integrins are the main receptors for extracellular matrix proteins like collagen, fibronectin and laminin. Cell–matrix interaction via integrins is essential for embryonic development, proliferation, survival, adhesion, differentiation, migration of cells, etc. [17–21].

Ligand binding to the extracellular integrin domain induces conformational changes and integrin clustering for activation of signalling cascades and recruitment of multiprotein complexes to FAs [13,22]. Subsequently, kinase activity-lacking integrins transmit messages through a variety of intracellular protein

Table 1
Current or completed (M=on the market) clinical trials targeted to integrins

Agent	Commercial name	Company	Type of agent	Target	Disease	Trial status
mAb						
HU23F2G, 23F2G	LeukArrest	Icos	mAb, chimeric human-mouse (IgG4)	CD11, integrin β2, integrin αLβ2 (LFA-1)	ischaemic stroke	phase III
	Erlizumab	Genentech, Roche	mAb, human-mouse light chain dimer	human CD18, integrin αLβ2 (LFA-1)	ischaemic stroke, acute myocardial infarction	phase II
	Abciximab	Centocor	mAb	integrin αvβ3	Angina pectoris	M
M200	Volociximab	Protein Design Labs	mAb	integrin α5β1	renal cell carcinoma metastases	phase II
M200	Volociximab	Protein Design Labs	mAb	integrin α5β1	melanoma metastases (combination with DTIC)	phase II
MEDI-525		MedImmune Inc.	mAb	integrin αvβ3	refractory advanced solid tumours, Leukemia, Lymphoma, small intestine cancer	phase I
MEDI-522	Vitaxin	MedImmune Inc.	mAb	integrin αvβ3	metastatic malignant melanoma	phase II
MEDI-522	Vitaxin	MedImmune Inc.	mAb	integrin αvβ3	rheumatoid arthritis	phase II
MEDI-522	Vitaxin	MedImmune Inc.	mAb	integrin αvβ3	metastatic androgen-independent prostate cancer, combination therapy	phase II
MEDI-522	Vitaxin	MedImmune Inc.	mAb	integrin αvβ3	plaque psoriasis	phase II
MLN01 (LDP-01)		Millenium Pharmaceuticals	mAb	integrin α2	cardiovascular diseases, thrombosis, stroke	phase I/II
MLN02		Millenium Pharmaceuticals	mAb, humanized	integrin α4β7	Crohn's disease	phase II
MLN02		Millenium Pharmaceuticals	mAb, humanized	integrin α4β7	ulcerative colitis	phase II
Small molecule blocker						
Ro 27-2441		Hoffmann-la Roche		dual integrin antagonist	asthma	phase II
Ro 27-2771		Hoffmann-la Roche		dual integrin antagonist	asthma	phase II
	Thalidomide		small molecule agent	integrin αv and β3 promoter	malignant gliomas	phase II
Peptide						
BIO-1211		Biogen, Merck and Co	peptide	integrin α4β1 (VLA-4)	allergy, asthma	phase II
EMD121974	Cilengitide	EMD Pharmaceuticals, Merck KGaA	peptide	integrin αvβ3 and αvβ5	Kaposi's sarcoma	
EMD121974	Cilengitide	EMD Pharmaceuticals, Merck KGaA	peptide	integrin αvβ3 and αvβ5	renal cell carcinoma, colon cancer	phase I
EMD121974	Cilengitide	EMD Pharmaceuticals, Merck KGaA	peptide	integrin αvβ3 and αvβ5	glioblastoma multiforme	phase II
EMD121974	Cilengitide	EMD Pharmaceuticals, Merck KGaA	peptide	integrin αvβ3 and αvβ5	first recurrence of glioblastoma multiforme	phase II
EMD121974	Cilengitide	EMD Pharmaceuticals, Merck KGaA	peptide	integrin αvβ3 and αvβ5	newly diagnosed glioblastoma multiforme	phase I/II
EMD121974	Cilengitide	EMD Pharmaceuticals, Merck KGaA	peptide	integrin αvβ3 and αvβ5	unresectable stage III or stage IV melanoma	phase II
EMD121974	Cilengitide	EMD Pharmaceuticals, Merck KGaA	peptide	integrin αvβ3 and avβ5	advanced solid tumours or lymphomas	phase I
EMD121974	Cilengitide	EMD Pharmaceuticals, Merck KGaA	peptide	integrin αvβ3 and αvβ5	acute myeloid leukaemia (AML)	phase II
eptifibatide	Integrilin	Millenium Pharmaceuticals	cyclic heptapeptide containing KGD sequence	integrin α2β3	acute coronary syndrome	M
Nonpeptide						
Tirofiban	Aggrastat		synthetic compound, nonpeptide, mimic of RGD	integrin α2β3	Acute coronary syndromes	M
TR-14035, SB683698		Tanabe Seiyaku, GlaxoSmithKline	nonpeptide	integrin α4β1 (VLA-4), integrin α4β7 (LPAM-1)	asthma, inflammatory bowel disease, multiple sclerosis	phase II

kinases and adaptor molecules such as ILK, FAK, talin, paxillin, parvins, p130Cas, Src-family kinases and GTPases of the Rho family (Fig. 1) [12,14,23–25]. As shown for leukocytes, additional cytoplasmic pathways impacting on integrin function are activated by cytokines and growth factors. These routes have been shown to increase chemotaxis or aggregation involving phosphatidylinositol 3-kinase, RHO (RAS homologue), RAP1, RAPL and DOCK2 (dedicator of cytokinesis 2), and LFA1 (lymphocyte function-associated antigen 1) avidity [26]. The current model of integrin activation involves the recruitment of talin to the cytoplasmic domain of β integrins whereby unbound integrins expressed on the cell surface are primed and transferred into an activated status [27–29]. The localization and activation of talin to the plasma membrane is enhanced by PIPKIγ (phosphatidylinositol phosphate kinase type-Iγ). Talin binds through its FERM (Four point one, Ezrin, Radixin, Moesin) domain to the small cytoplasmic domain of β integrins, thereby disrupting a salt bridge between α and β subunit for increasing integrin affinity, interactions with the ECM and linkage to the cytoskeleton [30–32]. Recent data, however, questioned the exact role of the salt bridge in vivo [33].

FA formation at the cytoplasmic face of the cell membrane includes the connection of adaptor proteins to the cellular actin cytoskeleton as well as the connection of integrins with receptor tyrosine kinases (RTKs). For example, talin, α/β/γ-parvin, α-actinin, vinculin and a MIG2/kindlin-2–migfilin–filamin complex with F-actin and PINCH and Nck2 link signals from integrins, EGFR and PDGFR (platelet-derived growth factor receptor) [28,34].

Integrin overexpression or loss contributes to several diseases, including squamous cell carcinomas and other tumours [35–39]. Changes in integrin expression patterns differentially affect tumour invasion and metastasis. For example, upregulation of α6β4 integrins has been reported in skin and head and neck tumours and might account for an increased adhesion of tumour cells in the process of metastasis. On the other hand, reduced integrin levels promote cell detachment from the primary tumour and invasive growth. A reduced α2, α3 and α5 integrin expression was found in several

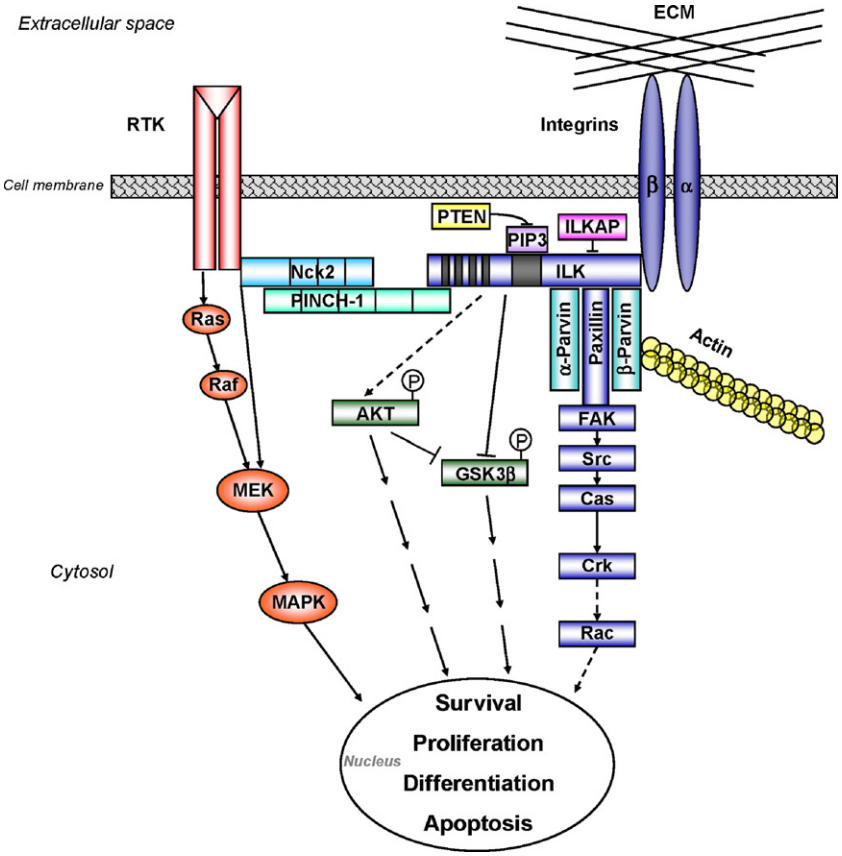

Fig. 1. The integrin-actin-RTK network. Cooperative integrin-receptor tyrosine kinases (RTK) signalling determines survival, proliferation, differentiation and apoptosis of cells. A main structural and signalling protein involved is ILK (integrin-linked kinase). ILK binds integrins, the LIM-only proteins PINCH-1 and -2, α-, β-, γ-parvin, paxillin and PIP3 (phosphatidylinositol-3,4,5-trisphosphate). Via PINCH and Nck2 it connects integrin and growth factor signalling and via parvins the ECM to the cytoskeleton. Kinase activity of ILK is PI3K-dependent and involves binding of PIP3 to the central pleckstrin homology domain. ILK phosphorylates AKT on serine 473 in tumour cells and inhibits GSK3β (glycogen synthase kinase-3β) through phosphorylation on serine 9. ILK activity is negatively regulated by ILKAP (ILK-associated protein serine/threonine phosphatase of the PP2C family), and PTEN (phosphatase and tensin homologue deleted on the chromosome 10). The tyrosine kinase FAK (focal adhesion kinase) localizes to focal contacts through binding to paxillin. FAK phosphorylation on tyrosine 397 offers a binding site for Src, which in turn leads to activation of Src, phosphorylation of FAK and an increased affinity to p130Cas, paxillin and Crk. Crk binding to phosphorylated p130Cas mediates Rac activation and results in formation of lamellipodia and subsequent migration of the cell. Different MAPK (mitogen-activated protein kinase) pathways can be initiated by RTKs, including a pathway via the adaptor protein Nck2.

carcinomas [17,40,41]. By promoting invasion and metastasis, integrins have been shown to substantially influence the prognosis of epithelial tumours [42–44]. Overall rarely observed, a heterozygous point mutation in the I-like domain of the β1 integrin subunit, T188I, has been found in SCC4 human cancer cells derived from a squamous cell carcinoma of the tongue [45]. These cells are poorly differentiated with only 1% of cultured cells expressing the terminal differentiation marker involucrin [46]. The T188I point mutation did not alter overall integrin expression levels, but exerted constitutive activation of integrin ligand binding, promoted cell spreading and led to sustained activation of ERK1/2 (extracellular signal-regulated kinase-1/2). Differentiation of the SCC4 cells could be increased by introduction of the wild-type β1 subunit [46]. These data demonstrated that mutations in integrins might affect tumour differentiation, invasion and progression. Screening of 124 human oral squamous cell carcinomas to identify tumour-specific sequence variations in β1 integrins revealed several single nucleotide changes in the DNA encoding the I-like domain of β1 integrin. The same mutations were also present in normal tissue of the patients. One mutation led to a change in amino acid sequence, which did not result in reduced β1 integrin expression or constitutive integrin activation and is also unlikely to affect the structure of the I-like domain [47]. It would be interesting to compare the frequency of these mutations with a control collective to assess whether they reflect normal polymorphisms or may contribute to the development of squamous cell carcinomas.

Elegant studies with breast cancer cells have demonstrated that the addition of inhibitory anti-β1-integrin antibodies or the re-expression of α2β1 integrins leads to the reversion of the malignant phenotype in 3-dimensional cell culture and to a reduction in tumour formation in vivo [48,49]. Overexpression of a β4 integrin mutant in hepatocellular carcinoma cells resulted in a down-regulation of α6β1 integrin expression. These cells displayed impaired adhesion, migration and invasion. In addition, they had slower growth rates and reduced anchorage-independent growth [50]. A study using β1 integrin double knockout lymphocytes and retransfection of β1 integrin deletion mutants showed that different parts of the cytoplasmic domain of β1 integrin are required either for adhesion or for invasion and metastasis [51]. The vital function of β1 integrin in embryonic development depends on the presence but not the phosphorylation of the evolutionally conserved tyrosine motif NPxY in its cytoplasmic tail [52]. Sakai et al. [53] demonstrated that the phosphorylation of specific cytoplasmic amino acid residues of β1 integrins are controlled by v-Src. The v-Src-dependent oncogenic transformation found in these cells was accompanied by defective cell adhesion, spreading and migration. Moreover, integrins can have complex roles in tumour growth e.g. by inhibition of angiostatin production as shown in α1-null mice [54].

2.1. ILK-mediated integrin signalling

ILK was first described in 1996 as an ubiquitously expressed serine/threonine kinase that binds directly to the cytoplasmic domain of the β1 integrin subunit [12]. The gene encoding ILK has been mapped to human chromosome 11p15.4/15.5 and encompasses 13 exons and 12 introns [55,56]. The 50 kDa ILK protein consists of 452 amino acids and exhibits three functional domains with distinct functions.

The amino-terminal domain contains four ankyrin (ANK) repeats, which are essential for binding to the LIM adaptor proteins PINCH-1 and PINCH-2 [57], ILKAP (ILK-associated protein serine/threonine phosphatase of the PP2C family) [58] and localization of ILK to focal adhesions (Fig. 1). The interaction between ILK and PINCH-1 was identified by a yeast two-hybrid screen with the N-terminal ILK ANK-repeat domain [57]. The two isoforms PINCH-1 and PINCH-2 consist of five LIM domains and tandem nuclear localization signals [59]. For binding to ILK all four ankyrin repeats of ILK are required, but only the N-terminal LIM domain of PINCH-1 or PINCH-2 specifically interacts with ILK in a mutually exclusive manner [57,60,61].

The central ILK PH (pleckstrin homology) domain was reported to be activated by PIP3 (phosphatidylinositol 3,4,5-triphosphate) in rat IEC-18 intestinal epithelial cells [62]. The carboxy-terminal kinase catalytic domain of ILK mediates structural interactions with β integrin subunits, with the actin-binding proteins α-, β- and γ-parvin, thereby providing a connection to the actin cytoskeleton [63–66], and with the paxillin LD1 motif (Fig. 1) [67,68].

Assembly of the ILK–PINCH–parvin complex is essential for its localization to FAs [69–72]. Interestingly, the binding between ILK and PINCH and to a lesser extent between ILK and parvin stabilizes these proteins and protects them from proteasome-mediated degradation [28,69].

The central role of the ILK–PINCH complex and the integrin–actin connection in cellular function is underlined by recent studies. For example, overexpression of the N-terminal domain of ILK which includes the PINCH binding ankyrin repeats or the ILK-binding LIM1 domain of PINCH leads to disruption of the complex resulting in reduction of fibronectin matrix deposition, cell adhesion, spreading, motility and proliferation as well as increased apoptosis [73–75]. The identification of the PINCH-interacting protein Nck2 [34] elucidated a connection between integrin- and growth factor receptor-mediated signalling pathways as Nck2 recognizes growth factor receptors such as EGFR and PDGFR through its carboxyterminal SH2 domain (Fig. 1) [76,77]. Binding of Nck2 to PINCH-1 is accomplished by the LIM4 domain of PINCH and the SH3 domain of Nck2 [34,78,79].

Except for its structural functions, ILK is discussed as serine/threonine kinase phosphorylating AKT on serine 473 and GSK3β on serine 9 in a PI3K (phosphatidylinositol-3-kinase)-dependent manner [62,80,81]. The PI3K-regulating tumour suppressor phosphatase PTEN (phosphatase and tensin homologue deleted on the chromosome 10) and ILKAP negatively regulate ILK kinase activity [58,82,83]. However, ILK-independently, DNA-PK (DNA-dependent protein kinase) directed to AKT serine 473 has been isolated from lipid rafts of plasma membrane and from the membrane fraction of HEK293 cells [84,85]. Additionally, ILK-deficient mouse

chondrocytes and fibroblasts still phosphorylate AKT on serine 473 [86,87] and the expression of ILK-mutants with reduced ILK kinase activity rescued the ILK-deficient phenotype in worms and flies [88–90]. Because most of the studies showing ILK-dependent phosphorylation of AKT were done in tumour cells, it was suggested that AKT activation is more dependent on ILK in tumour cells [91].

A second ILK downstream target, GSK3β, is involved in cell cycle regulation through GSK3β-dependent cyclin D1 proteolysis and AP-1-dependent transcriptional activation [92,81]. Additional regulation of cyclin D1 expression is mediated by ILK itself: Translocation of β-catenin to the nucleus is promoted by ILK and leads to transcriptional activation of cyclin D1 through Lef/Tcf (lymphoid enhancer factor/T cell factor) [93]. ILK also regulates the expression of the epithelial adhesion protein E-cadherin by downregulation of its promoter activity in $APC^{-/-}$ human colon carcinoma cells [94]. Besides these severe pathological effects, ILK plays an essential role in tumour angiogenesis through regulation of VEGF expression and blood vessel formation [95], in regulating of iNOS (inducible NO-synthetase) in macrophages involving AKT and NF-κB (nuclear factor κB) [96] and for activation of the NF-κB pathway by HER2/neu [97]. In response to hypoxia, hsp90 (heat shock protein 90) contributes to stabilization of ILK expression, which in turn controls the expression of SDF-1 (stromal cell derived factor-1) and ICAM-1 (intercellular adhesion molecule-1) in a NF-κB- and HIF-1α (hypoxia-inducible factor-1α)-dependent manner [98].

Another gene that seems to be transcriptionally regulated by ILK is encoding for MMP9 (matrix metalloproteinase 9). Increased expression of ILK upregulates MMP9 transcription through activation of AP-1 transcription factor [99]. The authors could show that inhibition of ILK activity in human glioblastoma cells and in ILK-overexpressing mammary epithelial cells significantly perturbed invasion into laminin-rich basement membrane (lrBM) Matrigel. Similar conditions also revealed a dependency of cell migration on ILK [100].

ILK transcription itself is stimulated by the transcription factor PPARβ (peroxisome proliferator-activated receptor β) through binding to a PPAR response element located in intron 2 of ILK in keratinocytes. Transcriptional activation of ILK and also PDK1 by PPARβ in turn activates AKT and promotes survival of these cells [101].

The cell culture experiments mentioned clearly demonstrate a central role of ILK in cell survival. In addition, animal experiments showed that ILK is necessary for embryonic development and that the function of ILK cannot be replaced by other proteins. ILK deficiency in *Drosophila melanogaster* and *Caenorhabditis elegans* caused embryonic lethality due to muscle attachment defects [88,90]. Similarly, ILK knockout in mice caused embryonic lethality at embryonic day 5.5–6.5 due to defective epiblast polarization and cavitation associated with abnormal F-actin accumulation at sites of integrin attachments to the basement membrane zone [28,87]. Interestingly, the ILK kinase-deficient E359K mutant localized to FAs and rescued defects in ILK null mice [68,87,88,90]. This ILK mutant has been previously reported to exhibit a dominant-negative phenotype with respect to kinase activity of ILK [82]. Subsequent work, however, has shown only slight reduction in kinase activity [66]. The mutant seems to mediate rather blocking of paxillin binding and localization to FAs than rescuing ILK function in ILK null mice [68].

A recent report adds to the already known ILK functions the regulation of cytoskeletal organization, cell morphology and migration through a ROCK (Rho-associated coiled-coil-containing kinase)-mediated pathway [102]. The serine/threonine kinase ROCK is a direct effector of RhoA, RhoB, and RhoC isoforms [103]. Rho-family GTPases including Cdc42, Rac1 and RhoA [104,105] are responsible for reorganization of actin cytoskeletal structures, like stress fibres, lamellipodia and filopodia, in response to integrin signalling [106]. These processes are necessary for cell spreading and cell motility. In detail, Cdc42 and Rac1 are inevitable for the formation of filopodia and lamellipodia, respectively, and RhoA mediates contractility, focal adhesion and stress fiber formation [105]. The RhoA/ROCK pathway involves inhibition of myosin phosphatase and activation of the LIM kinase/cofilin pathway in regulating actin cytoskeleton formation [107]. ILK signalling essentially contributes to this regulation of cellular cytoskeletal organization and migration. Overexpression of wild-type ILK suppresses cell spreading, polarization, and motility as shown in osteosarcoma cells. In this model, inhibition of RhoA or ROCK and constitutive activation of Rac1 reversed ILK-dependent effects on spreading, motility and morphology. The stable expression of the dominant negative E359K ILK mutant, which fails to bind paxillin and to localize to focal adhesions, potentiated cellular motility and reversed downstream RhoA/ROCK signalling. The increased spreading of E359K ILK cells can be suppressed by active RhoA or negative Rac1 [102]. Furthermore, ILK was shown to activate Rac1 via the β-PIX exchange factor in fibroblasts and endothelial cells [108].

Since ILK is involved in the regulation of cell survival, it is obvious that ILK plays a role in cancer. Expression analysis demonstrated upregulation of ILK in several types of cancer, including colon cancer, breast cancer, prostate cancer, malignant melanoma, and non-small cell lung cancer, often in a stage- and grade-dependent manner and associated with metastatic events [109–112]. ILK overexpression in breast epithelial cells was sufficient to induce mammary gland hyperplasia and tumour formation in transgenic mice [113]. These data suggest upregulated gene transcription, increased translation, elevated mRNA or protein stability of ILK in tumour cells, offering ILK as a target for therapeutic approaches. Surprisingly, immunohistochemical staining of biopsies from head and neck squamous cell carcinomas and colorectal carcinomas against ILK clearly showed that ILK expression is lower in undifferentiated and increased in more differentiated areas of the tumour (Haase, Cordes, 2006, unpubl. observ.). This observation suggests that ILK is not uniformly associated with a high proliferation rate and/or oncogenic transformation.

In summary, the central structural and signalling protein ILK serves as a link between the extracellular matrix and the cytoskeleton via integrins, paxillin and parvins and, together with the adaptor proteins PINCH and Nck2, between integrins and receptor tyrosine kinases [15,114]. Additionally, it activates a range of signalling pathways through its kinase activity.

2.2. The role of FAK in integrin signalling

The non-receptor bound tyrosine kinase FAK is involved in mediating both integrin and RTK signalling for the regulation of adhesion, cell shape, and cell motility.

FAK was first described in 1992 as a substrate of the Src oncogene and reported as a highly phosphorylated protein that colocalizes to integrins and FAs [115–117].

The 125 kDa protein FAK is ubiquitously expressed and consists of an N-terminal FERM domain, a central kinase domain, three proline-rich regions and a C-terminal FAT (focal adhesion targeting) domain. The FERM domain mediates interaction with RTKs, for example EGFR and PDGFR, with the non-receptor tyrosine kinase ETK (endothelial/epithelial tyrosine kinase) and with the actin- and membrane-associated protein ezrin [118–120]. The FERM domain can also be post-translationally sumoylated at position lysine 152 by a small ubiquitin-related modifier, SUMO, which is associated with nuclear translocation and catalytic activation of FAK [121]. This implicates a potential role of FAK in transferring signals between plasma membrane and nucleus.

The two proline-rich regions near the C-terminus of FAK provide binding sites for SH3 (Src homology 3) domains. Binding of the SH3-domain-containing adaptor protein p130Cas (Crk-associated substrate) promotes cell migration through activation of Rac (Fig. 1) [122,123].

The C-terminal FAT region of FAK is responsible for binding of ILK- and integrin-associated proteins like paxillin and talin and for localization of FAK to integrins and FAs [124,125]. In addition, the FAT domain binds directly to p190RhoGEF, a RhoA-specific guanine nucleotide exchange factor, and over-expression of FAK promoted p190RhoGEF tyrosine phosphorylation and RhoA activation, suggesting a link to Rho signalling pathways [126].

FAK activation occurs after external integrin-, growth factor- or G-protein-linked stimuli and starts with autophosphorylation at tyrosine 397 either in an inter- or intramolecular manner followed by recruitment of Src-family kinases and binding and phosphorylation of p130Cas and paxillin [106,125,127–129]. Src phosphorylates FAK at tyrosine 861, which increases the binding affinity of p130Cas to the proline-rich regions of the FAK C-terminus [130]. This SH3-mediated binding of p130Cas to FAK induces tyrosine phosphorylation of p130Cas at multiple sites, which in turn leads to SH2-mediated binding of Crk to p130Cas [122,123]. Paxillin phosphorylation on tyrosine 31 and tyrosine 118 by FAK-Src also enhances binding of Crk to paxillin [131,132].

Binding of the SH2 domain-containing adaptor protein GRB2/SOS (growth factor receptor bound 2/homologue of *Drosophila melanogaster* “son of sevenless” protein) to the FAK tyrosine 925 site seems to play a major role in activating the pro-survival Ras/Raf/MEK/MAPK pathway [125,133]. Phosphorylation of ERK2 in response to binding of GRB2/SOS to FAK activates the myosin light chain kinase, which promotes cell survival and proliferation [123]. FAK activity is also modulated by protein-tyrosine phosphatases, either in a positive or negative fashion [134,135].

FAK knockout mice suffer from defective embryonic morphogenesis and die at embryonic day 8.5. Fibroblasts derived from FAK null mice showed disturbed microtubule polarization and reduced migration. In contrast to the expectations, they also show increased formation of FAs [136–138]. These findings implicate that FAK is involved in disassembly of focal adhesion sites via regulation of Rho-family GTPases during migration of cells [106,137,139]. A recent study provides evidence that ILK communicates with FAK regarding RhoA/ROCK signalling [102], wherein FAK negatively regulates RhoA [139,140].

As mentioned above, overexpression of ILK in the U2OS osteosarcoma cell line suppresses cell spreading, polarization and motility. This also results in diminished FAK activation in response to fibronectin adhesion, possibly by perturbed autophosphorylation on tyrosine 397. In contrast, FAK over-expression in these cells rescued the ILK defects [102]. ILK seems to inhibit the integrin-mediated activation of FAK. Mechanisms of negative regulation of RhoA by FAK might be associated with FAK/Src-dependent phosphorylation of p190RhoGAP, subsequent association with p120RasGAP, GAP (Rho-GTPase activating protein) activity, and Rho inactivation [126,141], but the exact mechanisms remain to be elucidated.

FAK seems also critical for tumour progression. This is underlined by the observation that elevated FAK mRNA levels were found in human carcinomas and in acute lymphoblastic leukaemias [142,143]. Furthermore, the gene encoding FAK, termed PTK2 (protein-tyrosine kinase-2), is located at a locus (human chromosome 8q24) that is increased in number in tumour cells. This amplification may be responsible for upregulation of FAK protein expression [144]. FAK over-expression in cancer is discussed to contribute to an invasive phenotype due to increased formation of invadopodia [145,146]. Investigation of the special role of FAK in tumour progression and invasion should lead to a better understanding helpful to develop targeted therapies.

2.3. Nck as a link between integrin and growth factor signalling

Nck proteins belong to the SH2/SH3 adaptor proteins. There are two known family members with a molecular weight of 47 kDa, Nck1/Nck/Nckα [147] and Nck2/Nckβ/GRB4 (growth factor receptor binding protein 4) sharing 68% amino acid identity [148]. The *Drosophila* homologue is named Dock (Dreadlocks). Via their SH3 domains, Nck proteins can bind to phosphotyrosine residues of other proteins [149]. Both mammalian Nck proteins seem to be largely functionally redundant due to similar expression

patterns and the fact that mice deficient for either Nck1 or Nck2 are viable. However, because Nck1 does, in contrast to Nck2, not bind to PINCH, Nck proteins are not functionally redundant in this respect. Since the Nck2 knockout has no phenotype in mice, the integrin/ILK/PINCH-1/Nck2 association seems to have no essential role in embryonic development. In contrast, Nck1/Nck2 double knockout animals die at embryonic day 9.5 due to profound defects in mesoderm-derived notochord [148]. Fibroblasts isolated from the double knockout embryos have defects in cell motility and in lamellipodial actin network organization [150]. Recently, Nck adaptor proteins have been shown to participate in development of specialized cells important for proper glomerular function through interactions with tyrosine-phosphorylated YDxV binding motifs in the cytoplasmic tail of nephrin [151]. The 180 kDa transmembrane protein Nephrin functions as a structure and signalling protein in podocytes (kidney epithelial cells). The Nck–Nephrin interaction is important for actin reorganization in these cells. Targeted disruption of Nck in podocytes of transgenic mice results in defective formation of foot processes of the slit diaphragm in the kidney. Overall, nearly 30 interacting partners have been described for Nck proteins [152].

Both Nck proteins have a similar structure with three N-terminal SH3 domains and one C-terminal SH2 domain [34]. Since only Nck2 interacts with the LIM-only protein PINCH [34], connecting it to integrins via ILK, mainly Nck2 is discussed in this review. The SH2 domain-mediated interaction of Nck2 with FAK is dependent on phosphorylation of tyrosine 397, which is a site involved in the regulation of cell motility [153]. Overexpression of Nck2 decreases cell motility which may be mediated by the SH3 domains of Nck2 [153]. On the other side, Nck2, like Nck1, interacts with growth factor receptors or their substrates including EGFR, PDGFR-β, and IRS-1 [154]. The interaction with growth factor receptors is mainly facilitated by the SH2 domain, whereas the interaction with IRS-1 is mainly facilitated by the SH3 domains of Nck2 [34,154]. Although the functions of both Nck proteins seem to be mostly overlapping, overexpression of Nck2 but not Nck1 blocks PDGF-stimulated membrane ruffling and formation of lamellipodia due to binding to distinct sites at this receptor [155]. Whereas Nck2 binds to PDGFR-β at tyrosine 1009, Nck1 binds to tyrosine 751.

A number of reports indicate that Nck proteins are oncogenic. For example, upregulation of Nck2 in NIH 3T3 cells leads to focus formation [156]. In order to become invasive and metastasize, tumour cells need to develop invadopodia, actin-rich membrane protrusions with a matrix degradation activity. The formation of invadopodia is stimulated by EGF and is further promoted by neural WASP (N-WASP), ARP (actin-related protein) 2/3 complex, and their upstream regulators, Nck1, Cdc42, and WIP (WASP interacting protein) [157]. Cofilin is necessary for the stabilization and maturation of invadopodia. In the process of angiogenesis, VEGF induces the formation of FAs through Nck1 and PAK [158]. Both Nck1 and Nck2 cooperatively interact with v-Abl to transform NIH 3T3 cells and influence the morphology and anchorage-dependent growth of wild-type Ras-transformed cells [159]. Targeting of Nck proteins by cancer directed drugs should therefore influence tumour growth, anchorage dependence and growth factor signalling.

2.4. PINCH in integrin signalling

PINCH (particularly interesting new cysteine-histidine rich protein), also called LIMS (LIM and senescent cell antigen-like domain) is the name for two proteins, PINCH-1 [59] and PINCH-2 [160,61]. They contain five LIM-domains as their only known characteristic protein domains and are therefore called “LIM-only” proteins. LIM domains (first described in LIN-11, ISL1 and MEC-3) are protein–protein interaction domains with cysteine-rich zinc-finger structures that usually interact with tyrosine-containing motifs. The *Drosophila* homologue of PINCH is stck (streamer duck). It has a role in integrin-dependent epithelial cell adhesion, in muscle cell adhesion and actin-membrane anchorage. The *Caenorhabditis elegans* homologue of PINCH is unc-97 (uncoordinated movements clone 97). Unc-97 interacts with unc-98 in FA complexes. Mutation of unc-97 causes uncoordinated movements, as the name indicates.

Human PINCH-1 has a molecular weight of 37 kDa, consists of 325 amino acids and is located on chromosome 2q12.3–q13. PINCH-1 interacts with ILK via its first (N-terminal) LIM domain [57,161,162]. This N-terminal LIM domain is essential for targeting PINCH-1 to cell–matrix contact sites [75], stabilizing ILK expression and cell shape regulation and survival [163]. PINCH-1 and ILK are critical regulators of cell spreading [69] and, like α-parvin, crucial for cell survival [69]. The fourth LIM domain of PINCH-1 interacts with the third SH3 domain of Nck2, a complex suggested to function in cell shape and migration [163]. Rsu-1 (ras suppressor 1), which inhibits JNK activity [164] binds to the fifth LIM domain of PINCH-1 [165]. RNAi-mediated knockdown of Rsu-1 moderately inhibits cell attachment. A short 15 amino acid residue tail C-terminal to the fifth LIM domain is required for both cell shape modulation and survival [163]. Thymosin-β4 forms a complex with ILK and PINCH-1 in cardiac myocytes, which leads to the activation of AKT [166]. Besides a similarity in homology of 92% [160], PINCH-1 and PINCH-2 are similarly distributed in adult tissues. The fifth LIM domain of PINCH-2, which has a lower homology to PINCH-1, does not bind Rsu-1 [165].

Concerning embryonic development, PINCH-1 is inevitable for this process since loss of PINCH-1 causes embryonic lethality at the peri-implantation stage [70,167]. In contrast, knockout of PINCH-2 in mice produces no obvious phenotype, which probably results from overlapping functions with PINCH-1 [168]. PINCH-1/-2 double knockout fibroblasts have defects in cell spreading and adhesion.

In human cancers, PINCH-1 is upregulated in stromal cells, especially at the invasion front [169]. This finding suggests that PINCH-1 plays a role in promoting tumour–stromal interactions that support tumour progression. Indeed, PINCH-1 is an independent prognostic marker in colorectal cancer [170].

3. Integrins, integrin associated molecules and genotoxic injury

It has been described already in 1992 that integrins take part in the cellular reaction to genotoxic injury. Onoda et al. described that non-lethal irradiation of melanoma cells results in upregulation of αIIbβ3 integrin. This is associated with an increased adhesion to fibronectin and an enhanced rate of metastasis in a lung colony assay in vivo [171]. Confirmatory data were reported in a variety of normal and transformed human cell lines, which showed increased cell adhesion on the basis of radiation dose-dependent upregulation of β1, β3 and α5 integrins [172–175].

Integrins and adhesion of cells to ECM confer higher resistance to ionizing radiation and cytotoxic drug; a phenomenon known as CAM-RR and CAM-DR [173–184]. Recent data demonstrate a regulatory role of β1A integrins that transduce pro-survival signals to paxillin, p130Cas and JNK in a FAK-independent manner [177]. In a model of normal prostate epithelial cells, adhesion to laminin 5 via α6β4 integrin enhanced cell cycle arrest in the G2 phase after ionizing radiation [185]. Previously, it was shown that cell–ECM interactions prolong the radiation-induced G1 or G2 cell cycle blocks. This is thought to enable the cell to optimize the repair of the DNA [186,187] and seems at least partly to be responsible for CAM-DR and CAM-RR.

Induction of apoptosis in fludarabine-, etoposide- or bleomycin-treated human leukaemia cells was prevented by integrin-mediated adhesion to ECM on the basis of upregulation of Bcl-2-like proteins in parallel to downregulation of proapoptotic proteins such as Bax or Bim [179,181,188,189]. Adhesion of K652 chronic myelogenous leukaemia cells to fibronectin, mediated by α5β1 integrin, leads to apoptosis resistance after treatment with the BCR/ABL inhibitors AG957 and STI-571 as well as DNA-damaging drugs and γ-radiation [179].

The survival promoting effect of integrins has also been observed in the response of tumour to conventional chemotherapeutic agents [184] such as paclitaxel [190]. In a hepatoma cell culture model it was shown that the anti-apoptotic effect of integrins in response to a variety of chemotherapeutic agents was mediated by p42/p44 MAPK and p38 MAPK [191]. In cultured mouse endothelial cells grown on gelatine, type IV collagen, laminin or RGD peptides, activated integrins protected cells from bleomycin-mediated genotoxic injury [192]. Integrins also play a role in the tissue remodelling in chronic radiation damage. In a mouse model, knockout of integrin αvβ6 protects from radiation-induced lung fibrosis, probably because this integrin subtype interferes with activation of TGFβ1 [193]. Although these data in majority generated in two-dimensional cell cultures have provided useful knowledge, more complex cell culture systems such as spheroids and lrBM Matrigel assays eventually reflect the in vivo situation in a more physiological manner [194]. Early work from Durand [195–197] and Dertinger [198] highlighted the substantial impact of cell morphology and cell–cell communication for human tumour cells genotoxically injured with a diverse set of cytotoxic drugs such as 5-fluorouracil, doxorubicin, chlorambucil or mitomycin C or ionizing radiation. As shown in Fig. 2, cultivation of a selected set of human squamous carcinoma cells and normal human skin fibroblasts indicates severe morphological differences among each other and in comparison of 2D versus 3D (Deuse, Hehlgans, Cordes, 2006, unpubl. data). In general, growth of cells, normal or malignant, in 3D confers a tremendous reduction in radiation and drug sensitivity [197], an effect likely to be of utmost importance for tumour control and patient survival.

As suspected, not only integrins, but also integrin associated proteins modulate the radiation response of cells. Recently we found that tumour cells expressing high levels of constitutive active-kinase ILK exhibit increased radiosensitivity [199]. Consistent with these data, silencing of ILK expression in human A549 lung cancer cells results in significantly higher survival rates after irradiation an observation confirmed in ILK-transfected FaDu squamous cell carcinoma cells [180]. Besides adherent growing cells, this anti-survival action of ILK was detected in human HL-60 and Jurkat leukaemia cells [182]. Irradiation of leukaemia cell lines induced apoptosis by recruitment of caspase-8 or -9 by ILK in an adhesion-dependent manner. Vice versa, ILK downregulation leads to radioprotection and indicates an important role of ILK in the cellular radiation response. However, Gemcitabine-treated pancreatic carcinoma cells [200] after siRNA-mediated knockdown of ILK, thyroid cancer cells exposed to the potent ILK small kinase inhibitor QLT267 [201] or glioblastoma cells after ILK knockdown using antisense oligonucleotides [202] clearly demonstrated a survival-advantaging function of ILK. Currently, these differential effects upon ILK targeting might be best explained by the distinct cell death mechanisms induced by the different agents. Cytotoxic drugs as well as knockdown strategies predominantly activate apoptosis-related pathways that mostly affect short-term survival. From a radiobiological point of view, these examinations lack determination of clonogenic survival representing long-term survival, which evidently reflects the antisurvival power of a given agent most reliable as compared to apoptotic end points [203]. Further, the most relevant type of cell death upon irradiation of cells from solid tumours seems to be mitotic cell death and not apoptosis, which predominantly occurs in cells from the hematopoietic system and their malignant progeny [204].

In opposition to the radioprotection observed in $ILK^{-/-}$ cells, investigation in immortalized $PINCH\text{-}1^{-/-}$ mouse embryonic fibroblasts showed significant radiosensitization in contrast to $PINCH\text{-}1^{fl/fl}$ cells (Koch, Sandfort, Cordes, 2005, unpubl. observ.). Taking into account that knockdown of PINCH-1 or ILK also leads to proteasome-mediated degradation of its binding partners [69], these contrary effects on radiation survival are even more astonishing. The mechanisms leading to radioresistance in case of ILK knockdown and to radiosensitization in case of PINCH-1 knockdown, respectively, remain to be elucidated but suggest that the interaction between ILK and PINCH-1 are not as interdependent as suspected and/or are cell type-specific. Understanding the complex signalling network between these two proteins, their binding partners and

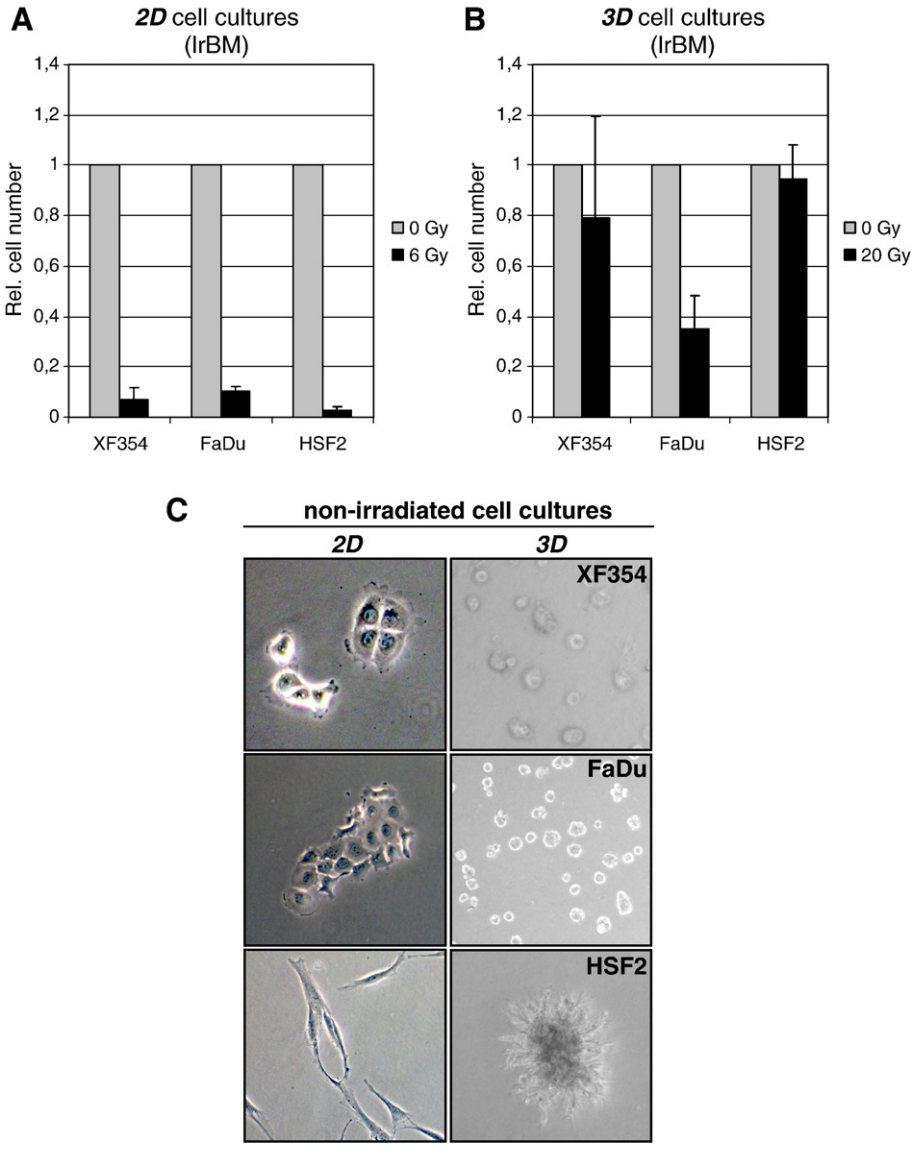

Fig. 2. Growth of malignant and non-malignant cell lines in three-dimensional laminin-rich basement membrane (lrBM) Matrigel tremendously reduces the cellular sensitivity to radiation-induced genotoxic injury relative to conventional two-dimensional cell culture conditions (A, B). Intriguingly, these data also pinpoint the heterogeneity of the cellular radiation survival response under 2D and more apparently under 3D. Human squamous cell carcinoma cells (XF354, FaDu) and normal human skin fibroblasts (HSF2) were irradiated with 6 or 20 Gy and relative cell numbers were determined after 8 to 11 days. Photographs illustrate the different morphologies of non-irradiated cell lines grown in a 2D-versus 3D-microenvironment (C, magnification, 20×).

the role of the ECM should provide insight into their relevance in treatment of tumour patients by ionizing radiation.

Only few studies deal with the influence of radiation on FAK expression or phosphorylation and evaluated FAK's role in resistance against therapeutic agents. The first study from Kasahara et al. revealed protection of FAK-overexpressing human HL-60 leukemia cells against radiation-induced apoptosis [205,206]. DNA fragmentation and activation of caspase-3 and -8 were significantly reduced in these cells compared to vector controls. In parallel, the PI3K/AKT survival pathway was activated and inhibitor-of-apoptosis proteins cIAP-2 (cellular inhibitor of apoptosis 2) and XIAP (X-linked inhibitor of apoptosis protein) were induced. Mutation analysis of FAK showed that the FAK autophosphorylation site tyrosine 397 in the central kinase domain as well as focal adhesion target regions, namely tyrosine 925, were essential for the anti-apoptotic function of FAK. Subsequent studies indicate resistance of FAK-over-expressing cells against TRAIL-(tumour necrosis factor (TNF)-related apoptosis-inducing ligand) induced apoptosis, whereas opposite effects were observed after siRNA-mediated down-regulation of FAK [205,206]. siRNA-mediated knockdown of FAK in pancreatic cancer cells increased the rate of anoikis and suppressed metastasization [207]. Smith et al. [208] showed that BL melanoma cells were sensitized to 5-fluorouracil by FAK antisense oligonucleotide transfection.

Following irradiation of A549 human lung carcinoma cells, Beinke et al. [209] showed increased phosphorylation of the FAK autophosphorylation site tyrosine 397, followed by enhanced interaction with Src and tyrosine 925 phosphorylation of FAK, whereas FAK expression was not affected. Phosphory-

lation of tyrosine 925 of FAK promotes binding of GRB2/SOS, which in turn activates the Ras/Raf/MEK/MAPK pathway. Additionally, phosphorylation of the FAK-binding partner paxillin on tyrosine 31 and tyrosine 181 was induced, as well as upregulation of paxillin expression 2 to 6 h after exposure of cells to 6 Gy X-rays. The downstream target of FAK, p130Cas, showed a higher amount of phosphorylation in response to irradiation. These findings suggest that the FAK-signalling pathway, including paxillin, Src, p130Cas, Rac and the survival pathway Ras/Raf/MEK/MAPK may play an important role in modulating cell fate after exposure to radiation.

Downstream of these proximal signalling molecules, it has been shown that CAM-DR is mediated by activation of the transcription factor NF-κB [210]. This study showed that the DNA binding heterodimer consists of RelB-p50, which is different from the binding complex after activation by TNFα (tumour necrosis factor alpha). For hematopoietic tumours, the cell cycle inhibitor $p27^{kip1}$ plays a role in CAM-DR by increasing the percentage of cells in G1 arrest [181,211]. That NF-κB is crucial for CAM-DR was demonstrated in normal and transformed mammary epithelial cells [212]. Resistance to apoptosis required β4 integrins expressing functional hemidesmosome targeting domains for proper development of cell polarity and NF-κB activation.

Further studies are necessary to dissect the mechanisms used by tumour cells and non-tumour cells to overcome CAM-RR and CAM-DR in order to design targeted therapies directed to specific signalling molecules. Fig. 3 illustrates non-comprehensively cytosolic signalling pathways that have so far been identified to be critically involved in pre-existent and/or acquired resistance of cells to genotoxic agents.

4. Perspectives and implications for anticancer strategies

This review has shown that integrin-associated proteins are involved in all major signal transduction pathways regarding

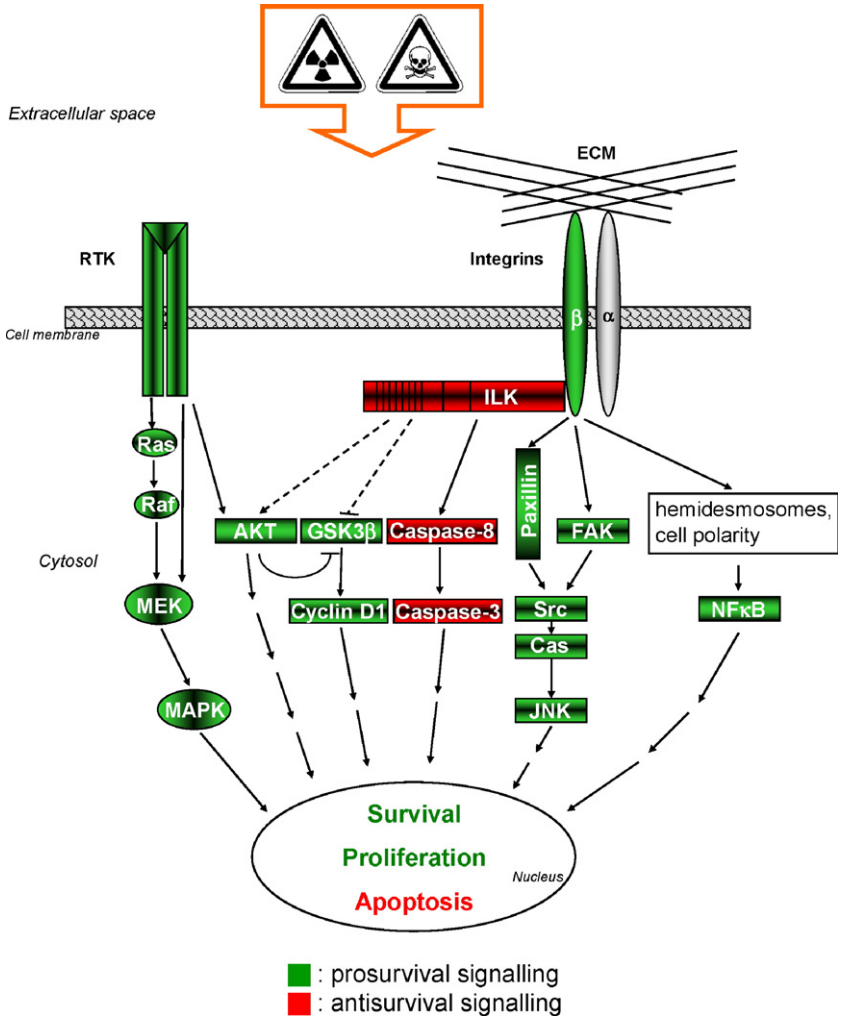

Fig. 3. This scheme summarizes integrin associated signalling pathways critical in determining the cell's response to genotoxic injury. Upon exposure of cells to cytotoxic agents, the cells are triggered by their microenvironment through integrin binding to ECM and growth factor binding to their cognate receptors whether to survive or to die. The indicated pathways are a small selection from a large number of molecules known to modulate the cellular reaction to external cell death-inducing stimuli. Integrin- and RTK-associated cascades such as Ras/Raf/MAPK, β integrin/FAK/JNK or β integrin/NFκB channel prosurvival (GREEN) messages, which appear cell type- and/or context-dependent. ILK, in contrast, seems to mainly mediate antisurvival (RED) signals via inhibition of AKT or apoptosis-promoting interactions with caspase-8.

proliferation and survival and are therefore likely candidates for targeted therapies (Fig. 3). Greater insight into the molecular mechanisms regarding integrin-, ILK-, and PINCH-1-mediated modulation of the cellular radiation and drug response should help to develop and optimize diagnostic as well as therapeutic strategies in tumour treatment.

In recent years, clinical studies with anti-integrin antibodies [213–215], growth factor receptor antagonists [216–219], and radiolabelled monoclonal antibodies [220] have been approved. A huge number of clinical trials based on integrin inhibition for treatment of diverse diseases such as inflammation and a variety of late stage tumours are under way or completed (Table 1). Specific targeting of αvβ3 and α5β1 integrins expressed on neovascular endothelial cells is of increasing importance to suppress tumour angiogenesis [221]. For example, Vitaxin, a humanized monoclonal antibody, which has specificity for the integrin αvβ3 (vitronectin receptor), interferes with blood vessel formation and has been tested in advanced leiomyosarcomas [222] as well as in various other advanced carcinomas, e.g. carcinomas of the breast, colon and kidney [223,224]. Cilengitide, a cyclic peptide mimicking the RGD motif, has led to partial remission of head and neck squamous cell carcinomas [225] increasing the cure rate from 15% to 53% in combination with radiotherapy in a nude mouse model [226]. This substance is in phase I and II trials in non-small cell lung cancer (as a monotherapy), pancreatic cancer (in combination with gemcitabine), glioblastoma [227] and in other advanced solid tumours [228]. The most selective nonpeptidic α5β1 antagonist SJ749 reduced proliferation of astrocytoma cell lines dependent on α5β1 expression levels and cell culture conditions, underlining the importance of α5β1 as a target for anticancer therapies [229]. Furthermore, a recently characterized anti-tumour protein, angiocidin, might inhibit angiogenesis through binding collagen and integrin α2β1 present on many tumour cells [230]. A 20 amino acid N-terminal peptide of angiocidin promoted α2β1-dependent adhesion of K562 cells, disrupted HUVEC (human umbilical vein endothelial cell) tube formation and inhibited tumour growth as well as angiogenesis in a mouse model.

To address β1 integrin as a therapeutic target, Park et al. [231] tested the β1 integrin inhibitory antibody AIIB2 in a number a breast cancer cell lines using the three-dimensional lrBM Matrigel model. They showed that β1 integrin inhibition resulted in decreased proliferation and increased apoptosis of cancer cells. A nonmalignant cell line, however, remained resistant. These cell-specific effects could be confirmed in vivo.

Taking into account the diversity in expression and activation of integrin- and growth factor receptor-associated signalling molecules, an increased understanding of the molecular differences between cancer cells and normal cells is likely to promote the development of therapies that specifically target cancer cells, including antibodies, RNA interference or small molecule inhibitors. Directed against tumour-associated antigens, these compounds could prove useful especially in synergism with other therapies such as radiotherapy and conventional chemotherapy. The specificity of such therapies might offer greater efficacy and less toxicity due to the conservation of normal tissue.

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