ROLE OF INTEGRIN SIGNALING IN CELL PROLIFERATION AND SURVIVAL

WENJIE BAO

Stockholm 2005

Division of Pathology, Department of Laboratory Medicine, Karolinska Institutet
Karolinska University Hospital-Huddinge, Stockholm, Sweden

ROLE OF INTEGRIN SIGNALING IN CELL PROLIFERATION AND SURVIVAL

WENJIE BAO

Stockholm 2005

Published and printed by US-AB

ISBN 91-7140-394-9

To Ming and Bilin
To my parents

ABSTRACT

Integrins function as transmembrane cell adhesion receptors for the surrounding extracellular matrix (ECM). Within the tissues, integrins attach the cells to the surrounding ECM and sense the ECM environment by triggering diverse arrays of signaling pathways that are essential for the control of many central cellular functions, including cell proliferation and survival. These cellular processes critically impact tumor development. Therefore, insights into detailed molecular mechanisms of integrin functions will contribute to the understanding of tumor progression, and may thereby provide novel strategies for tumor treatment.

The present study aimed to investigate: (I) the role of integrin signaling in the control of anchorage-dependent cell proliferation, particularly in regulating two cell cycle components; the cyclin-dependent kinase 2 (Cdk2)-inhibitors $p21^{CIP1}$ and $p27^{KIP1}$. (II) the role of integrin signaling regulating cell survival, in particular for integrin αv promotion of melanoma cell survival.

We firstly developed a method for analyzing specific integrin signaling-mediated responses independent of cell spreading by utilizing an agonistic, immobilized anti-integrin monoclonal antibody directed against the fibronectin receptor integrin α5β1. This method was then adopted as a part to examine the role of integrin signaling in the regulation of $p21^{CIP1}$ and $p27^{KIP1}$. We showed that integrin ligation led to a rapid degradation of $p21^{CIP1}$ and $p27^{KIP1}$, including proteasome-mediated degradation of $p21^{CIP1}$ in an ubiquitin-independent manner. Importantly, an integrin-activated small GTPase signaling pathway involving Cdc42 to Rac1 signaling was found to be functionally involved in regulating proteasomal degradation of $p21^{CIP1}$. Our results for the first time revealed that integrin signaling is capable of regulating protein stability and proteasomal degradation. Integrin-mediated proteasomal proteolysis might contribute to anchorage-dependent cell cycle control.

Integrin αvβ3 is a key adhesion receptor for melanoma cell survival and tumor growth in various models. While the tumor suppressor p53 is rarely mutated in malignant melanoma; it is still unclear why wt p53 is rendered non-functional in these tumors. Using a three-dimensional (3D) collagen gel model mimicking the dermal environment of malignant melanoma growth *in vivo*, we found that integrin αv promoted melanoma cell survival by inactivating p53 and thereby suppressed p53-induced apoptotic cell death. We also found that integrin αv controls melanoma cell survival in 3D-collagen through a pathway involving p53 regulation of MEK1 signaling. Surprisingly, we also came to the conclusion that integrin αv induced an unfolded, inactive wt p53 conformation within 3D-collagen. This finding reveals the first example of controlled regulation of wt p53 conformation in tumor cells *in lieu* of mutation, and may contribute to explain lack of need of p53 mutations in malignant melanoma. Thus, our results demonstrate that tumor cells can use an adhesion receptor to sense and respond to the surrounding 3D-environment by regulating the conformational states of a tumor suppressor. Importantly, $PRIMA\text{-}1^{MET}$, a small organic compound that can revert a mutant p53 to an active conformation, was also able to reactivate melanoma cell wt p53 conformation, induce apoptosis and suppress melanoma xenograft tumor growth in a wt p53-dependent manner. Therefore, our results point to a novel therapeutic approach against malignant melanoma based on restoration of wt p53 function.

In conclusion, we found an integrin-activated Cdc42 to Rac1 signaling pathway essential for proteasomal degradation of the Cdk2-inhibitor $p21^{CIP1}$. This may provide a novel mechanism in anchorage-dependent cell cycle progression. Furthermore, we found that integrin αv promotes melanoma cell survival through controlling the conformation, activation and apoptotic function of the tumor suppressor p53. By targeting wt p53 using a small organic compound, our studies also provide proof of principle for a new approach for development of malignant melanoma therapy. Such development is urgently needed given that present therapy is inefficient for metastatic malignant melanoma.

List of Publications

This thesis is based on the following papers, which will be referred to in the text by their roman numerals:

I. **Wenjie Bao** and S Strömblad (2002). Use of an immobilized antibody to examine integrin α5β1 signaling independent of cell spreading. *Biological Procedures Online* 4:81-87.

II. **Wenjie Bao**, M Thullberg, HQ Zhang, A Onischenko and S Strömblad (2002). Cell attachment to the extracellular matrix induces proteasomal degradation of $p21^{CIP1}$ via Cdc42/Rac1 signaling. *Molecular and Cellular Biology* 22:4587-4597.

III. **Wenjie Bao** and S Strömblad (2004). Integrin αv-mediated inactivation of p53 controls a MEK1-dependent melanoma cell survival pathway in three-dimensional collagen. *Journal of Cell Biology* 167:745-756.

IV. **Wenjie Bao**, N Issaeva, M Chen, K Wiman, G Selivanova and S Strömblad. Induction of an inactive wt p53 conformation by integrin αv points to a potential target for melanoma therapy. Manuscript.

Contents

1 ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
Apaf1	Apoptosis activating factor 1
ARF	Alternative reading frame ($p14^{ARF}$ in human, $p19^{ARF}$ in mice)
Cdk	Cyclin-dependent kinase
CDKI	Cyclin-dependent kinase inhibitor
CDKN2A	Cyclin-dependent kinase inhibitor 2A ($p16^{INK4A}$, MTS1)
ECM	Extracellular matrix
ERK	Extracellular signal-regulated kinase
FAK	Focal adhesion kinase
GEF	Guanine nucleotide exchange factor
Mab	Monoclonal antibody
MAPK	Mitogen-activated protein kinase
MEK	MAPK kinase
PI-3K	Phosphatidylinositol 3-OH kinase
PIX	PAK-interactive exchange factor
PRIMA-1	p53 reactivation and induction of massive apoptosis
PUMA	p53-upregulated modulator of apoptosis
Raf	MAPK kinase kinase
RGD	Arginine-Glycine-Aspartate
RGP	Radial growth phase
VGP	Vertical growth phase
wt p53	Wild-type p53
$\rightarrow$	Activates
$\dashv$	Inhibits

2 INTRODUCTION

Normal tissue cells continuously communicate with their surrounding environment (Geiger and Bershadsky, 2002). One of the important environmental factors is the extracellular matrix (ECM) that can interact with the integral membrane adhesion receptors, integrins, and activate a variety of signaling pathways leading to control of various cellular functions, including cell attachment, spreading, migration, invasion, differentiation, proliferation and survival. These cellular functions profoundly influence many physiological and pathophysiological processes, including embryonic development, tissue regeneration, wound repair, inflammation, angiogenesis and tumor progression.

2.1 THE EXTRACELLULAR MATRIX

The ECM is a highly organized, insoluble meshwork of macromolecules made up of three major components: fibrous elements (e.g. collagens, elastin and reticulin), adhesive glycoproteins (e.g. fibronectin, vitronectin, laminin), and space filling molecules (e.g. glycosaminoglycans covalently linked to proteins in the form of proteoglycans). The ECM constitutes the connective tissues of the body, including for example the different ECM types basement membrane, interstitial stroma and elastic connective tissue. The ECM is secreted, assembled and remodelled by the cells within or in contact with the ECM, and the ECM then surrounds and organizes the cells by providing a structural scaffold. The ECM is a key environmental factor that can exchange information with cells through cell surface receptors such as integrins, and contribute to the control of diverse cellular functions, including cell proliferation and survival (Bissell and Radisky, 2001).

2.1.1 **Extracellular matrix proteins**

Collagens are a family of fibrous glycoproteins composed of three collagen polypeptide chains, called α chains. Each α chain contains the repetitive motif Glycine-X-Y, in which X and Y can be any amino acid. The three α chains are warped around one another to fold into a rope-like, triple stranded helix (Gordon and Olsen, 1990; van der Rest and Garrone, 1991). Slightly varied collagen chains assemble and generate distinct types of collagens that either further polymerize to form collagen fibrils or promote network-formation with other ECM proteins. The collagens type I, II, III, V and XI are able to form fibers and are thereby referred to as fibrillar collagen (or fibril-forming collagens), which are the most abundant components of interstitial connective tissues as well as in elastic connective tissues like the dermis, and in ligaments, cartilage and bone (Kadler, 1994.). Collagens type IV, VI, VIII, X and XVIII are network-forming collagens, and these are the most abundant constituent of basement membranes (Knupp and Squire, 2003). The network-forming collagens can self-assemble and organize into a lattice-network within the lamina densa of the basement membrane.

Fibronectin is a large nonfibrous glycoprotein. The fibronectin monomer is built up by three types of repeating modules (type I, II and III) that allow fibronectin to bind to various molecules, including collagen, heparin, fibrin, glycosaminoglycans, fibronectin itself as well as integrin and syndecan cell adhesion receptors (Hynes, 1990). Fibronectin exists in insoluble multimeric fibrils in the ECM. Fibronectin not only plays a role in organizing the matrix, but also functions as an adhesive protein that helps cell to attach. In contrast to collagen assembly, the polymerization of fibronectin is driven by the cells (Schwarzbauer and Sechler, 1999).

The triplet sequence arginine-glycine-aspartate (RGD) motif is found in many ECM proteins, including in fibronectin, collagen, vitronectin, osteoponin and thrombospondin. This triplet sequence can be recognized by certain integrins, including integrin αvβ3 (Ruoslahti, 1996). Therefore, peptides containing an RGD motif can be used to block the integrin to ECM interactions (Ruoslahti and

Pierschbacher, 1986). In collagen, the present RGD motifs are normally cryptic and inaccessible. Under denatured conditions, however, the RGD-motif of type I collagen is exposed (Davis, 1992). Melanoma cell adhesion to denatured collagen type I was found to use integrin αvβ3 to recognize the collagen RGD motif (Davis, 1992). In a 3D-collagen model, melanoma cells were found to be able to degrade collagen type I leading to the exposure of cryptic integrin αvβ3-binding sequence (Montgomery et al., 1994).

2.2 INTEGRIN RECEPTORS

Integrin are principle cell adhesion receptors that mediate cell to ECM interactions. Integrins are a large family of heterodimeric transmembrane adhesion receptors, consisting of non-covalently bound α and β subunits (Hynes, 1992). In mammalian cells, the 18 α and eight β subunits combine in a restricted manner to form 24 distinct integrins with differing but overlapping ligand binding and functional properties (Hynes, 2002) (Figure 1). For example, the β1 subunit can pair with twelve different α subunits, and forming twelve distinct integrins with different ligand binding properties. Other integrin subunits, like β5, can only pair with one α–subunit (αv). Although most integrins mediate cell-ECM adhesions, certain integrins, including those with a β2-subunit, mediate cell-cell adhesion (Hynes, 2002).

2.2.1 **Integrin structure**

Integrin α and β subunits contain large extracellular (700-1100 residues), small transmembrane and short cytoplasmic domains (30-50 residues) (Figure 2).

The integrin extracellular domains

Integrin α subunits contain four or five domains. They are β-propeller, the 'inserted' (I), Thigh, Calf-1 and Calf-2 domains. At the N-terminal region, seven repeats of about 60 amino acids fold into a single domain, called the β–propeller. In half of integrin α subunits, including α1 and α2, a module of about 200-residues is inserted between repeat 2 and 3 of the β–propeller domain, this insert is called I domain, and also referred to as von Willebrand factor-type A domain (Heino, 2000; Humphries, 2000). I-domain contains a divalent cation-binding motif, called metal ion-dependent adhesion site or MIDAS motif, which is proposed to play a key role for a metal-dependent integrin-ligand interaction and ligand binding specificity in the I-domain containing integrins (Springer and Wang, 2004). Beneath the integrin α subunit β–propeller domain, there is a large portion of the α subunit extracellular domain of about 500 amino acids. The crystal structure shows presence of three β–sandwich domains in this region, called the thigh, calf-1 and calf-2 domains (Takagi and Springer, 2002).

The integrin β subunit contains four domains. One of them is the I-like domain that has detectable sequence homology to the I domain present in certain α subunits (Takagi and Springer, 2002). The I-like domain contains two adjacent metal coordination sites, the ADMIDAS (adjacent to MIDAS) and LIMBS (ligand-associated metal binding site) (Xiong et al., 2001; Xiong et al., 2003).

The extracellular I- and I-like domains are major ligand-binding sites, allowing integrins to specifically bind to their ligands in both a conformation- and a divalent cation-dependent manner (Springer TA, 2004). In I-domain containing integrins (e.g. α1 and α2 integrins), ligand binding is mediated by the I-domains. In other integrins (e.g. αv integrins), ligand binding is mediated by a β–propeller domain from the α subunit in combination with the I-like domain in the β subunit (Arnaout, 2002).

Changes in integrin affinity states of activation may be coupled to conformational changes within the integrin extracellular domain, shifting between a bent and an extended shape (Takagi and Springer, 2002).

The cytoplasmic domains

Integrin cytoplasmic tails are much shorter than the extracellular domains; however they can affect the structure and function of the extracellular domains. To this end, the cytoplasmic tails can regulate conformational changes and thereby control integrin activation states and binding affinity for the ligands, a phenomenon called 'inside-out signaling' (Ginsberg et al., 1992). Upon ligand binding, the cytoplasmic domains mediate integrin interaction with the cytoskeleton and thus transduce numerous signaling into the cells, a process called outside-in signaling.

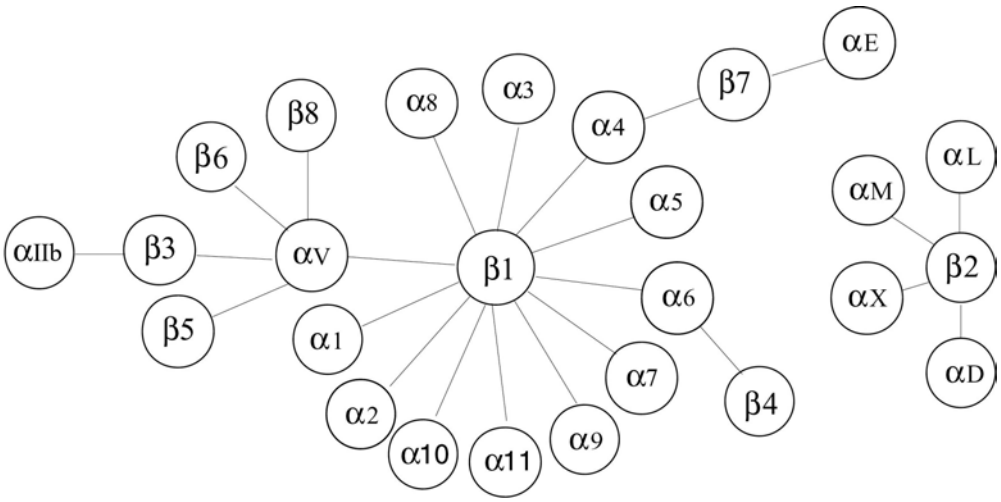

Figure 1 In human, 18 integrin α subunits and 8 β subunits together form 24 distinct heterodimers, differing in their ligand binding specificities. The scheme shows the 24 different integrin combinations.

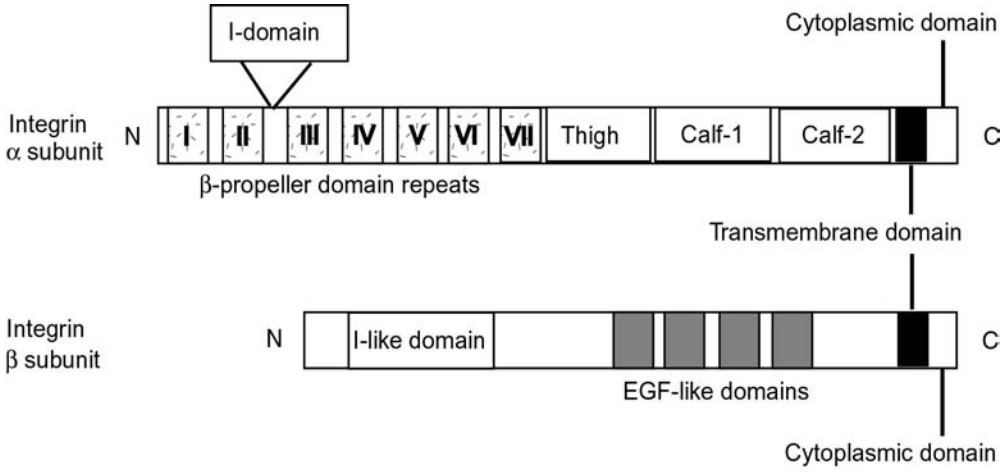

Figure 2 The domain structure of integrin α and β subunits, showing the locations of I and I-like domains in their respective subunits.

2.2.2 **Integrin-mediated cell-matrix adhesions**

Using integrins, cells sense and respond to their surrounding ECM environment (Miranti and Brugge, 2002). Distinct integrins can bind to either a specific ECM or several ECM proteins (Hynes, 2002). For example, at least eight integrins, including integrin $\alpha5\beta1$, can bind to FN, and at least five integrins, including integrin $\alpha6\beta4$, bind to laminin. The $\beta1$-integrins $\alpha1\beta1$, $\alpha2\beta1$, $\alpha10\beta1$ and $\alpha11\beta1$ are the common receptors for native collagens (Heino, 2000) while denatured or degraded collagen can also be recognized by integrin $\alpha v\beta3$ (Davis, 1992). Part of this thesis is focused on the function of integrin $\alpha v\beta3$, which can bind a number of ECM proteins, including fibronectin, fibrinogen, vitronectin, osteopontin and proteolysed collagen type I.

When cells anchor to ECM, integrin engagement leads to formation of membrane-associated protein complexes at sites called cell-matrix adhesions (Zamir and Geiger, 2001). Cell-matrix adhesions are able to provide dynamic bi-directional linkages between the ECM and the intracellular cytoskeleton. These membrane-spanning links play critical roles in regulation of ECM assembly, rearrangements of the cytoskeleton, transduction of signaling as well as in the control of cell morphogenesis, migration, proliferation, differentiation and survival (Hynes, 2004).

Cell-matrix adhesive structures

Different types of adhesive structures in which integrins intracellularly link to actin filaments, are known to include focal complexes, focal adhesions and fibrillar adhesions with different molecular compositions (Zamir and Geiger, 2001). Hemidesmosomes are a separated class of cell-matrix adhesive structures where integrins are connecting basement membrane on the outside of the cell to the intracellular keratin intermediate filaments (Garrod, 1993).

Focal complexes are formed when cells start to spread and form lamellae. They are small (approx. 1μm), dot-like adhesions present at the edges of the lamellipodia. The formation of focal complexes is induced by the small GTPase Rac1. Focal complexes serve as the precursors of focal adhesions (Geiger et al., 2001).

Focal adhesions are relatively well-characterized adhesion structures (Geiger et al., 2001). They are flat, elongated structures and are several square microns in area. Focal adhesions locate near the periphery of cells, anchor actin-filament bundles and mediate strong adhesion to the substrate. So far, focal adhesions are known to contain more than 50 distinct components and the small GTPase RhoA is promoting the formation of focal adhesions. Cytoskeleton proteins including vinculin, talin, paxillin and tyrosine phosphorylated proteins such as FAK are present in these protein complexes. Integrin $\alpha5\beta1$ and $\alpha v\beta3$ are adhesion receptors mostly found in focal adhesions.

Fibrillar adhesions are elongated or dot-like structures along actin stress fibers that mostly associate with soft fibronectin fibrils and are located in the centre of the cells (Geiger et al., 2001). Fibrillar adhesions are formed when integrin drives movement on lamellae towards the cell body. The formation of fibrillar adhesions leads to the stretch of cell surface fibronectin and formation of long fibronectin fibrils.

In fact, *in vivo* adhesion events also occur in three-dimensions (3D), where cell adheres to 3D mesh-like fibers rather than two-dimensional (2D) rigid substrates. A new type of cell-matrix adhesions—3D-adhesions is defined when cells adhere to a pliable 3D-environment (Cukierman et al., 2001), which may be similar to cell-matrix adhesions present in vivo. It should be noted that the cell-ECM adhesions found in 3D environments clearly differs from those typically observed in 2D. For

example, the 3D adhesions generally appear to be smaller (Cukierman et al., 2002). The characterization of 3D-adhesions will be discussed in section 2.7.1.

Hemidesmosomes are found in the epidermis and provide stable adhesion of epithelial cells to the underlying basement membrane (Garrod, 1993). It is known that the extracellular domain of integrin α6β4 mediates cell adhesion to laminin, and that the intracellular domain indirectly binds to keratin intermediate filaments. Hemidesmosomes are proposed the sites for integrin α6β4–triggered singling events (Borradori and Sonnenberg, 1999).

2.3 INTEGRIN SIGNALING

Integrins lack intrinsic enzyme activity. However, cytoplasmic tails of integrins associate with numerous adaptor proteins that link integrins to cytoplasmic kinases and transmembrane growth factor receptors that all possess kinase activity. Thereby, integrin engagement is able to trigger diverse intracellular signaling events, including the activation of focal adhesion kinase (FAK), Src-family kinases, integrin-linked kinase (ILK), phosphatidylinositol 3-OH kinase (PI-3K), protein kinase C, Ca^{2+}, different types of mitogen-activated protein kinases (MAPKs) ((extracellular signal-regulated kinases (ERKs), c-Jun NH2-terminal kinase (JNK) and p38 MAPK)), and the Rho family of small GTPases Cdc42, Rac1 and RhoA (Giancotti and Ruoslahti, 1999). Below, I will focus on describing three signaling pathways FAK, MEK/ERK1/2 and the small GTPases, which are closely correlated with my present study.

2.3.1 **FAK signaling**

FAK is one of abundant protein-tyrosine kinases at focal adhesions (Guan et al., 1991; Hanks et al., 1992; Schaller et al., 1992). Upon integrin clustering, FAK is recruited to focal adhesions and co-localized with integrins. A recent study showed that FAK could associate with integrins through the integrin-associated proteins talin and paxillin (Schlaepfer et al., 2004). FAK functions as a signaling mediator that recruits and interacts with SH2-domain- and SH3-domain-containing proteins, leading to transduction of numerous signaling pathways. FAK signaling is critically involved in the control of various cellular functions, including cell adhesion, spreading, migration, proliferation and survival (Parsons, 2003). Here, I will selectively describe a couple of FAK-pathways identified upon integrin activation.

Integrin engagement causes autophosphorylation of FAK at Tyr397 (Schaller et al., 1994), which creates a binding site for SH2-domain-containing proteins, mainly Src-family kinases (Calalb et al., 1995). When a FAK/Src signaling complex is formed, the scaffold protein paxillin is bound to FAK and phosphorylated by Src at Tyr31 and Tyr118. Then paxillin together with the docking protein p130CAS that binds to FAK after Src activation leads to recruitment and activation of the scaffold proteins Crk and Nck (Richardson and Parsons, 1996; Schlaepfer and Hunter, 1998). The signaling involved in this pathway branches and activates downstream signaling pathways, regulating different cellular functions. For example, the FAK→Src→paxillin pathway critically controls the turn over of focal adhesions (Wozniak et al., 2004).

The FAK→Src→p130CAS→Crk pathway results in recruitment of DOCK180 (human counterpart of the C.elegans gene *mbc* and *ced-5*, an exchange factor for Rac)/ELMO complex that was shown to stimulate GTP-loading of Rac1 (Brugnera et al., 2002). This pathway may provide a mechanism for activation of the small GTPase Rac1 at focal adhesion (Parsons, 2003).

The activated Src kinase also phosphorylates FAK at Tyr925, allowing docking of Grb2 leading to activation of a key signaling pathway from FAK to Grb2→ Ras→ mSOS (ganosine 5'-triphosphate exchange factor, GEF) → Raf (MAPK kinase kinase) → MEK (MAPK kinase) → ERK1/2 (Cobb and Goldsmith, 1995; Hill and Treisman, 1995; Marshall, 1995; Schlaepfer and Hunter, 1998). This pathway may be involved in the control of cell proliferation and survival.

2.3.2 **The Rho family of small GTPases**

The p21-Rho family belongs to the p21-Ras superfamily of small GTPases. At least 20 Rho-proteins have been identified and are roughly divided into five groups, Rho-like, Rac-like, Cdc42-like, Rnd-like and RhoBTB subfamilies (Burridge and Wennerberg, 2004). The Cdc42, Rac1 and RhoA are the best known, 'classical' Rho GTPases.

Rho GTPases act as molecular switches and functionally switch intracellular signaling pathways by cycling between an inactive GDP- and an active GTP-bound conformation (Raftopoulou and Hall, 2004) (Figure 3). The guanine nucleotide exchange factors (GEFs) stimulate the formation of active GTP-bound GTPases, while the GTPase-activating proteins (GAPs) promote GTPase inactivation by stimulating GTP-hydrolysis activity. The guanine nucleotide dissociation inhibitors (GDIs) are proposed to function as inhibitors of GTPase activation by inhibiting the dissociation of GDP from the inactive GTPase. Below, I will expand the discussion of the GEF family.

The GEFs is a large family. The largest group of GEFs is the Db1 family where around 60 members are found that serve as GEFs for the Rho family of the GTPases RhoA, Rac1 and cdc42 (Schmidt and Hall, 2002). The Db1 members typically contain the Db1-homology (DH) and pleckstrin homology (PH) domains tandem repeat (Hart et al., 1991). The PIX family (PAK-interactive exchange factors) is a newly identified member of the Db1 family of GEFs. PIX/PAK complexes enrich in Cdc42- and Rac-1-induced focal complexes and can mediate Cdc42 to Rac1 signaling (Manser et al., 1998). Because all the members of Db1 family contain PH domain, many of them may bind to phospholipids. Moreover, an additional family of Rho GEFs, the DOCK180/ELMO lacking DH-PH tandem domains, is identified as Rac GEFs involved in the p130CAS→Crk pathway (Kiyokawa et al., 1998a) described in section 2.3.1.

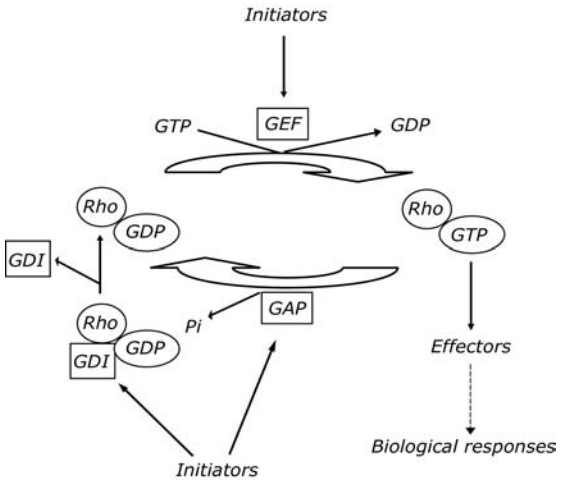

Figure 3 A biochemical model of regulation of activity of the Rho family of small GTPases. The cycle between the active, GTP-bound, and the inactive, GDP-bound, Rho GTPases is regulated by three classes of regulators: guanine nucleotide exchange factors (GEFs); GTP-activating proteins (GAPs); and guanine nucleotide dissociation inhibitors (GDIs). The extracellular signals (initiators) modulate these three regulators and affect the Rho GTPase activity, and active Rho GTPases can ineract with distinct effectors, resulting in various biological responses.

Integrin engagement is critically involved in activation of these small GTPases. Integrin ligation can rapidly activate Cdc42 and Rac1 (Price et al., 1998). Integrin-mediated cell adhesion can also activate Rho in an actin cytoskeleton-independent manner (Ren et al., 1999). Anchorage-dependent phosphorylation of p130CAS on tyrosine results in its association of an SH2/SH3-containing adaptor protein Crk and the recruitment of DOCK180, leading to Rac activation (Kiyokawa et al., 1998b).

The p21-activated protein kinases (PAKs) are a family of serine threonine kinase, which can function downstream effectors of Cdc42 and Rac1 (Bar-Sagi and Hall, 2000). PAK1 plays a role in controlling cytoskeleton organization and cell migration (Sells et al., 1997; Zhang et al., 2002). Anchorage-mediated activation of PAK1 is associated with the Rac1 and the SH2/SH3-dapter protein Nck (Bagrodia and Cerione, 1999; del Pozo et al., 2000; Li and She, 2000). However, PAK activation can also occur in the absence of Cdc42 or Rac activation by PIX (Obermeier et al., 1998).

Rho signaling pathways often crosstalk with distinct oncogenic signaling cascades, including those downstream of Ras (Malliri and Collard, 2003). Integrin signaling mediated by Ras superfamily-proteins include an activation of signaling through ERK (Grb2→ Ras → mSOS → Raf → MEK → ERK pathway, as mentioned in section 2.3.1).

Rho GTPases controls various cellular functions, including cytoskeleton reorganization, cell adhesion, spreading, morphology, migration, proliferation and malignant transformation (Jaffe and Hall, 2002; Malliri and Collard, 2003). Rho A induces the formation of focal adhesions and assembly of stress fibers (Ridley and Hall, 1992). Cdc42 promotes filopodia formation (Kozma et al., 1995) and controls polarity signals directing cell migration (Allen et al., 1998; Nobes and Hall, 1999). Rac1 is required for the formation of actin-rich lamellipodia protrusion that is proposed to be a main driving force for cell movement (Ridley and Hall, 1992).

The role of Rho family of the small GTPases in the control of the G1-cell cycle progression will be discussed in section 2.4.2.

2.3.3 **MEK/ERK1/2 signaling**

The extracellular signaling-regulated kinase 1/2 (ERK1/2) is a type of mitogen-activated protein kinases (MAPKs), and is activated commonly by oncogenic stress. Importantly, integrin engagement also activates ERK1/2 signaling cascades which control a variety of cellular functions, including gene expression, cell proliferation, differentiation and survival (Howe et al., 2002; Nishida and Gotoh, 1993).

Integrin-mediated activation of ERK1/2 requires other signaling, including Raf/MEK1, PAK1 and/or FAK (Howe et al., 2002).

Cell attachment-dependent ERK1/2 activation requires Raf1/MEK1 signaling. Integrin-mediated cell adhesion is required for phosphorylation of Raf1 and activation of MEK1, and MEK1 mediated activation of ERK1/2 (Chen et al., 1996; Renshaw et al., 1997). Cell attachment may also be required to couple Ras activation to MEK1 and ERK1/2 activation (Lin et al., 1997b; Renshaw et al., 1997). The integrin activated Ras→Raf→MEK1→ERK1/2 signalling cascade triggers a wide cellular response.

Cell attachment-dependent ERK1/2 activation also requires another important mediator, PAK1. The ability of PAK1 to regulate ERK1/2 may be due to a direct phosphorylation of Raf1, leading to MEK1 to ERK1/2 activation (Frost et al., 1997; King et al., 1998). The activity of PAK1 is suggested

to be involved in the formation of MEK1/ERK1/2 complexes and ERK1/2 activation in newly adhering cells (Eblen et al., 2002). In addition, upon cell adhesion, PAK1 can mediate MEK1 phosphorylation and subsequent activation of ERK1/2 (Slack-Davis et al., 2003).

The involvement of FAK in cell attachment-mediated ERK1/2 activation is controversial. Integrin-mediated transient activation of ERK1/2 was shown to be dependent on FAK (Chen et al., 1994). Upon cell attachment, autophosphorylation of FAK results in formation of the p130CAS/Shc/Grb2/SOS complex (Polte and Hanks, 1997; Schlaepfer et al., 1994; Schlaepfer and Hunter, 1997; Vuori et al., 1996), which may link the Ras→Raf→MEK1→ERK1/2 cascade because SOS signaling is a guanine nucleotide exchange factor for Ras activation (Egan and Weinberg, 1993; Khosravi-Far et al., 1995). However, others reported that FAK is not involved in activation of ERK1/2 because overexpression of constitutively active FAK failed to activate ERK1/2 (Frisch et al., 1996a). In addition, overexpression of dominant negative FAK in fibroblast prevented FAK activation, but failed to inhibit ERK1/2 activation by cell attachment to fibronectin (Lin et al., 1997a). Therefore, both FAK-dependent and –independent pathways may activate ERK1/2.

Integrin-mediated cell adhesion regulates ERK1/2 nuclear translocation and phosphorylation of its downstream transcriptional targets, including Elk-1, leading to immediate-early gene induction (Aplin et al., 2001).

The role of ERK1/2 signaling in the control of cell proliferation and survival will be discussed in section 2.4.2 and 2.4.4, respectively.

2.4 INTEGRIN CONTROL OF CELL PROLIFERATION AND SURVIVAL

Anchorage to the ECM is required for proliferation and survival of all normal tissue cells. As the major cell adhesion receptors for the ECM, integrins play key roles in these processes (Ruoslahti and Reed, 1994).

2.4.1 **Anchorage-dependent G1-cell cycle progression**

In order to control cell proliferation, anchorage regulates key cell cycle components during cell cycle progression (O'Neill et al., 1986). Here, I will focus on the effects of anchorage on the G1-phase of the cell cycle.

Progression through the G1-phase is controlled by the sequential activation of cyclin-dependent kinases (Cdks), in association with their regulatory subunits, cyclins. D-type cyclins, including cyclin D1 and D2, form active complexes with Cdk4 and Cdk6, and cyclin E1 and E2 form complexes with Cdk2. Cyclin D-Cdk4/6 and cyclin E-Cdk2 complexes are the main driving factors for the cell cycle progression through the G1-phase (Sherr and Roberts, 1999).

The activities of cyclin-Cdk complexes are regulated through the binding of two classes of cyclin-dependent kinase-inhibitors (Cdk-inhibitors or CDKI). The INK4CDKI family members, including $p15^{INK4b}$, $p16^{INK4a}$, $p18^{INK4c}$ and $p19^{INK4d}$, specifically inhibit cyclin D-Cdk4/6 activity by direct interaction with and sequestration of Cdk4/6 (Sherr and Roberts, 1995). The CIP/KIP family members, including $p21^{CIP1}$ and $p27^{KIP1}$, bind to cyclin D-Cdk 4/6 and cyclin E-Cdk2 complexes. At higher levels, $p21^{CIP1}$ and $p27^{KIP1}$ function as cyclin E-Cdk2 inhibitors, leading to cell cycle arrest. However, at lower levels, $p21^{CIP1}$ and $p27^{KIP1}$ actually promote the assembly, stability and nuclear retention of cyclin D-Cdk4 and cyclin D-Cdk6 complexes (LaBaer et al., 1997). Therefore, the relative

levels of cyclin, Cdk and CDKI proteins dictate whether a cell will progress through the G1 phase (Coleman et al., 2004).

Cell anchorage to ECM is required during the G-1 phase firstly for induction of cyclin D and activation of Cdk4 and Cdk6, and secondly for promotion of cyclin E-Cdk2 activity (Fang et al., 1996; Gad et al., 2004). Cell attachment to the ECM results in reduction of the Cdk2-inhibitors $p21^{CIP1}$ and $p27^{KIP1}$, whereas cells in suspension display higher levels of $p21^{CIP1}$ and $p27^{KIP1}$ (Gad et al., 2004; Strömblad et al., 1996; Zhu et al., 1996). In suspension, Cdk2 associates with $p21^{CIP1}$, leading to an impaired cyclin E-Cdk2 activity concomitant with a block of the cell cycle in G1-phase (Fang et al., 1996; Strömblad et al., 1996; Zhu et al., 1996). It appears that anchorage-dependent regulation of $p21^{CIP1}$ is important for proper control of cyclin E-Cdk2 activity.

2.4.2 **Control of G1-cell cycle components by integrin signaling**

Anchorage-activated integrin signaling, including FAK, MAPKs (ERK and JNK), PI-3K, ILK and Rac1, play key roles in the control of cell cycle progression of the G1-phase (Giancotti and Ruoslahti, 1999). These signaling components converge to regulate the activities of Cdks and the expression of cyclin D1. For example, integrin-mediated sustained ERK1/2 activation was shown to promote cyclin D1 expression in growth factor-treated fibroblasts (Roovers et al., 1999). In addition, upon integrin ligation, active Rac1 promotes cyclin D1 synthesis and thereby controls cell cycle progression through the G1-phase (Mettouchi et al., 2001), while Rho appears to maintain the correct timing of cyclin D1 expression in the G1-phase (Yamamoto et al., 1993).

The small GTPases Ras and Rho are also important regulators of $p21^{CIP1}$ and $p27^{KIP1}$. Overexpression of an active Ras mutant induced $p21^{CIP1}$ while Rho signaling suppressed the induction of $p21^{CIP1}$ and thereby promoted DNA synthesis (Olson et al., 1998). RhoA has been implicated to control the mid-late G1-phase and to downregulate $p27^{KIP1}$ (Hirai et al., 1997).

2.4.3 **Proteasomal proteolysis in cell cycle**

The proteasome pathway is a major intracellular proteolytic mechanism that controls various cellular processes, including the cell cycle. Ubiquitination targets proteins for proteasomal proteolysis and involves the coordinated actions of an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and an E3 ubiquitin-protein ligase. The last one delivers multiple ubiquitin molecules to the target protein and forms the ultimate polyubiquitin chain that serves as the signal for recognition and proteolysis by the 26S proteasome unit (Hochstrasser, 1996). The 26S proteasome is composed of a barrel-shaped 20S catalytic chamber, capped at each end by 19S complexes (Hershko and Ciechanover, 1998). A number of cell cycle components are targeted by proteasomes, including cyclins (A, B, D, E), E2F, Rb, p53, $p21^{CIP1}$ and $p27^{KIP1}$ (Blagosklonny et al., 1996; Boyer et al., 1996; Hofmann et al., 1996; King et al., 1996; Koepp et al., 1999; Pagano et al., 1995; Scheffner et al., 1993). The proteasome-mediated proteolysis of these components critically determines the periodicity of the cell cycle.

The transcriptional regulation of $p21^{CIP1}$ has been extensively studied (Gartel and Tyner, 1999). However, the regulation of $p21^{CIP1}$ levels by post-transcriptional mechanisms is still elusive. The $p21^{CIP1}$ protein has a short half-life, and is commonly degraded by the proteasome (Blagosklonny et al., 1996; Cayrol and Ducommun, 1998; Sheaff et al., 2000; Touitou et al., 2001).

In most cases, proteasomal degradation is preceded by ubiquitination. For example, the polyubiquitination of $p27^{KIP1}$ mediated by a SCF (Skp/Cull/F-box protein)-ubiquitin-ligase complex leads to degradation of $p27^{KIP1}$ by the proteasome (Pagano et al., 1995). However, although $p21^{CIP1}$

can be ubiquitinated, proteasomal degradation of $p21^{CIP1}$ does not require ubiquitination (Sheaff et al., 2000). It has also been shown that the C8 α-subunit of the 20S proteasome can directly interact with the C-terminus of $p21^{CIP1}$, leading to rapid degradation of $p21^{CIP1}$ (Touitou et al., 2001). Consistently, a recent report indicated that disruption of essential components of the ubiquitination mechanism did not influence endogenous $p21^{CIP1}$ degradation (Chen et al., 2004). However, it has been unclear if integrin-mediated anchorage to the ECM might regulate proteasome-dependent proteolysis.

2.4.4 **Control of cell survival by integrin signaling**

Apoptosis

Apoptosis is a form of programmed cell death and is an intrinsic cellular self-destructive suicide process essential for the control of tissue development and homeostasis. Defects in apoptotic pathways promote expansion of neoplastic cells, and enhance the escape of tumor cells from surveillance by the immune system, leading to tumorigenesis. Defects of apoptosis also cause cancer cell resistance to chemotherapy and irradiation.

Apoptosis is a highly regulated, stepwise process, starting with disruption of cellular membranes, following by a break of the cytoplasmic and nuclear skeletons, extrusion of the cytosol, degradation of the chromosomes and fragmentation of the nucleus. Eventually, the shrunk cell corpse is engulfed by neighboring cells or macrophages in the tissue and disappears typically within 24 h (Wyllie et al., 1980).

At least two apoptosis-inducing pathways have been well characterized. The intrinsic pathway is mediated by mitochondria upon intracellular stress and/or deprivation of survival factors. The extrinsic pathway is initiated by membrane death receptors activated by extracellular factors (Ashkenazi and Dixit, 1998). Both pathways involve the activation of cysteine aspartyl-specific proteases known as caspases that cleave their cellular substrates important for the cell and genome integrity, leading to the characteristic morphological and biochemical changes of apoptosis (Green, 2000).

Upon cellular stress, mitochondria release a number of apoptotic factors, such as cytochrome c, Smac/DIABLO (Second Mitochondrial Activator of Caspase) and HtrA2 (Jiang and Wang, 2004; Wang, 2001) promoting the intrinsic apoptotic pathway. Cytochrome c promotes apoptosis activating factor 1 (Apaf1) and initiator caspase 9, resulting in the formation of a complex known as the apoptosome, which triggers downstream caspase cascades including cleavage and activation of the executor caspase-3, and -7. Smac/DIABLO bind to and antagonize inhibitors of apoptotic proteins and thereby promote apoptosis (Suzuki et al., 2001; Wang, 2001). In fact, the change of mitochondrial membrane permeability is a central event, tightly controlled by the Bcl-2 family members, including proapoptotic proteins Bad, Bax, Bak, Bid, Bim, Noxa, and PUMA (p53-upregulated modulator of apoptosis) and the antiapoptotic proteins Bcl-2, Bcl-XL, and Bcl-W. These proteins either reside in or can translocate to mitochondria.

The extrinsic apoptotic pathway is typically activated by ligation of transmembrane death receptors including tumor suppressor factor receptor (TNFR), CD95 (FAS) and death receptor 5 (DR5) with their respective corresponding ligand TNF, FASL and TRAIL-R (TNF-related apoptosis inducing ligand-receptors 1). Upon ligand binding, these receptors promote the formation of death-inducing signaling complexes that lead to the activation of membrane-proximal initiator caspases including caspase-8 and -10, and these in turn cleave and activate executor caspase 3 and -7. In fact, there is extensive cross-talk between the intrinsic and extrinsic apoptotic pathways, which is proposed to lead to amplification of the apoptotic signals (Fulda et al., 2001).

In addition to caspase-dependent apoptosis mechanism mentioned above, apoptosis can also occur in the absence of caspase activity (Chipuk and Green, 2005).

The tumor suppressor p53 is a critical regulator of both intrinsic and extrinsic apoptotic pathways. The p53-mediated apoptosis mechanism will be discussed in section 2.6.1.

Control of apoptosis by integrin signaling
Anchorage to ECM is required for survival of many types of normal cells, including epithelial cells, and loss of anchorage leads to cell death by apoptosis (Frisch and Francis, 1994; Meredith et al., 1993). Importantly, not only loss of anchorage but also lack of specific integrin-mediated contact to the ECM can lead to apoptosis, although the cells are still firmly attached by other cell to ECM contacts (Montgomery et al., 1994; Petitclerc et al., 1999). To this end, integrin-activated signaling pathways, including FAK, PI-3K/Akt and MEK1/ERK1/2, can control both cell survival and apoptosis (Howe et al., 1998).

Overexpression of constitutively active FAK could prevent epithelial cell apoptosis (Frisch et al., 1996b). Inhibition of FAK triggered apoptosis in several types of cells (Hungerford et al., 1996; Xu et al., 1996). The mechanisms for FAK control of cell survival and apoptosis may be associated with PI-3K (Chen and Guan, 1994) whose downstream target Akt is critical for adhesion-dependent survival pathway by suppressing apoptosis (Khwaja et al., 1997). Integrin-activated FAK may also promote cell survival by repressing p53-dependent apoptosis (Ilic et al., 1998).

In addition, integrin-activated ERKs may regulate adhesion-dependent survival of epithelial cells (Frisch and Screaton, 2001). Upon integrin ligation, active ERK1/2 translocates to the nucleus where it activates a number of transcriptional genes that can promote cell survival (Schulze et al., 2001). In addition, integrin $\alpha v\beta 3$ mediates a sustained activation of MEK1/ERK1/2 in endothelial cells, which may promote cell survival during angiogenesis (Eliceiri et al., 1998). Moreover, integrin-mediated activation of ERK1/2 may promote anchorage-dependent melanocyte cell survival (Conner et al., 2003).

As mentioned above, specific integrins are required for suppression of apoptosis in certain cell types and situations. Mechanistically, integrins $\alpha 5\beta 1$ and $\alpha v\beta 3$ binding to fibronectin, are efficient in upregulating Bcl-2 (Matter and Ruoslahti, 2001; Zhang et al., 1995b), an antiapoptotic protein that can protect cells against apoptosis. Recently, integrin $\alpha 5\beta 1$-mediated cell attachment was shown to promote cell survival through the inhibition of a Bit1/AEX complex -mediated mitochondrial apoptosis independent of caspase activity (Jan et al., 2004). However, other integrins, including the fibronectin receptor integrin $\alpha v\beta 1$, do not provide this survival effect. Moreover, integrin $\alpha v\beta 3$ mediates endothelial cell survival associated with inhibition of p53 activity (Strömblad et al., 1996) as well as activation of the transcription factor nuclear factor kappa B (Scatena et al., 1998). Moreover, integrin $\alpha v\beta 3$ elevated the Bcl-2 to Bax ratio and promoted glioma, melanoma and endothelial cell survival (Petitclerc et al., 1999; Strömblad et al., 1996; Uhm et al., 1999). Overexpressed unligated integrin $\alpha v\beta 3$ may associate with activate caspase 8 in epithelial cells, leading to a death-receptor-initiated apoptosis (Stupack et al., 2001).

2.5 MECHANISMS OF CANCER

Today, more than a hundred distinct types of cancers are found in the human being, implicating the diversity and complexity of cancer. While cancer is a genetic disease associating with mutagenic alterations of oncogenes and tumor suppressive genes especially at the initial tumorigenic stage,

transformation of normal cells into cancerous derivatives must include multistep alterations. Recently, a number of essential alterations in cells were characterized as a self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. These six steps that constitute the uncontrolled tumor growth are suggested as the hallmarks of cancer, were carefully reviewed by Hanahan and Weinberg (Hanahan and Weinberg, 2000).

2.5.1 **The hallmarks of cancer**

Cancer cells acquire a capacity for self-sufficiency. This was the first of the six capabilities to be defined, in large part because of the prevalence of dominant oncogenes (Ras) that were found to modulate mitogenic growth signals. Thereby, cancer cells became independent on exogenous growth factors and less relying on their surrounding microenvironment, because they can manipulate various own factors for growth stimulation through different molecular strategies. For example, cancer cells switch the types of integrin receptors that they express, benefiting ones that transmit pro-growth signals that can activate the SOS→Ras→Raf→MAPK mitogenic cascade (Aplin et al., 1998; Giancotti and Ruoslahti, 1999; Lukashev and Werb, 1998). In contrast, normal cells are extremely dependent on extracellular growth signals to proliferate.

Cancer cells also acquire a capacity of insensitivity to anti-growth signals (e.g. TGF-β) and thereby can grow out of control, leading to a disruption of the tissue homeostasis. For example, in many different types of cancers the pRb pathway, as governed by TGF-β, is disrupted, which leads to release of E2Fs and promotion of cell proliferation (Fynan and Reiss, 1993). In contrast, normal cells sense the external stimuli and are able to correctly decide to proliferate, to be quiescent or to enter into a postmitotic, differentiated state.

In addition, cancer cells acquire the capacity of evading apoptosis, whereas normal cells will undergo apoptosis upon a variety of stimuli, including DNA damage, oncogenic stimuli and hypoxia or upon lack of extracellular stimuli from growth factors or ECM. Mutations in the *TPp53* gene and defects in the p53 pathway are the most common causes for cancerous cells to become resistant to apoptosis (Vogelstein et al., 2000).

Moreover, cancer cells acquire a capacity of limitless replicative potential, and when propagated in culture the cells appear to be capable of multiplying without limit — immortalization. In contrast, normal cells in culture have the capacity of approximately 60-70 doublings, a limited replication state, before becoming senescent.

Cancer cells also acquire the capacity of sustained angiogenesis in which new blood vessels are formed from pre-existed vessels. Angiogenesis is tightly regulated by a balance between positive and negative signals, including from soluble growth factors such as vascular endothelial growth factor (VEGF), cell adhesion receptors such as integrins, or negative signals, such as thrombospodin-1. Integrin αvβ3 is known to be critical for the control of angiogenic endothelia cell survival (Brooks et al., 1994; Strömblad and Cheresh, 1996).

Finally, cancer cells acquire the capacity of invasion and metastasis. Most types of tumor cells are able to move out of their original sites, invade to adjacent tissues and travel to distant sites where they may settle down. This process is called metastasis and is the cause of nearly all human cancer deaths (Sporn, 1996). Many adhesion molecules that mediate cell-cell adhesion and cell-ECM interactions are changed in cells achieving invasive or metastatic capacities. Despite their relative anchorage independence, cancerous cells still need integrins to promote growth, survival, migration and/or

metastasis (Bissell and Radisky, 2001; Wiseman and Werb, 2002). For example, integrin αvβ3 is critical for melanoma growth, invasion and metastasis (Hsu et al., 1998; Seftor, 1998). Moreover, the matrix-degrading proteases are aberrantly overexpressed and protease inhibitors are deregulated, which is associated with the capacity of tumor invasion and metastasis.

2.5.2 **Malignant melanoma**

Melanoma develops from malignant transformation of the pigment-secreting cells melanocytes. During embryonic development, melanocytes differentiate from neural crest progenitor cells and eventually reside in the basal epidermal layer mainly in the skin.

The proclivity for metastasis is a hallmark of melanoma, which leads melanoma to be the most common fatal cancer. For example, once cutaneous melanoma has progressed from the radial growth phase (RGP) to the vertical growth phase (VGP), the cure and survival rates of melanoma patients dramatically decline. Pathologically, most melanoma subtypes display a slow RGP, followed by a more rapid VGP (Clark et al., 1984) (See Figure 4). RGP melanoma cells extend upward in the epidermis but remain *in situ* and lack capacity to invade the dermis and metastasize, while VGP melanoma can invade the dermis and even deeper structures and metastasize to distant organs (Meier et al., 1998; Rusciano, 2000).

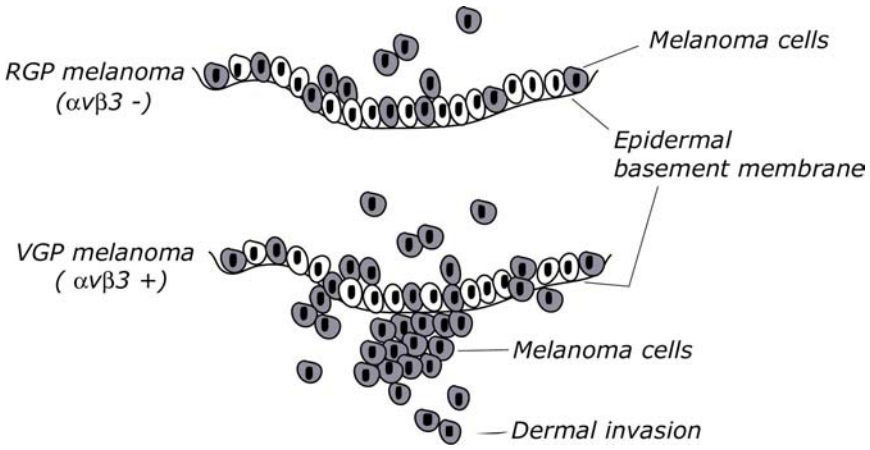

Figure 4 Schematic representation of melanoma growth at two stages: in situ melanoma, radial growth phase (RGP) melanoma that does not express integrin αvβ3; malignant melanoma, vertical growh phase (VGP) melanoma that expresses integrin αvβ3.

As in many cancers, environmental factors are proposed to highly influence the development of melanoma. Exposure of ultraviolet (UV) light appears to be a causal factor for melanoma genesis (Chin, 2003).

The genetic changes play key role for the development of melanoma. Through analyzing mutations or deletions in the melanoma genome, multiple genetic defects are found, including the inactivation of tumor suppressor genes. The tumor suppressor *cyclin-dependent kinase inhibitor 2A* (*CDKN2A*) on chromosome 9p21 appears to be associated with melanoma susceptibility in people with familial melanoma (Hussussian et al., 1994). The *CDKN2A* locus (INK4 locus) encodes two tumor suppressors,

$p16^{INK4a}$ (inhibitor of cyclin D/Cdk4 or Cdk6) and $p14^{ARF}$ ($p19^{ARF}$ in mice, stabilizing p53 by abolishing HDM2-induced p53 degradation), leads to suppression of both the INK4A/cyclin D/Cdk4/pRb pathway and the ARF/p53 pathway. These two pathways are known to be critical for tumor suppression through regulation of cell proliferation and apoptosis, respectively.

However, several melanoma pathological surveys indicated that an absence or very low incidence (< 5 %) of point mutation or allelic loss of *TP53* in surgical specimens of primary and metastatic melanomas (Satyamoorthy et al., 2001; Yang et al., 2001). This suggests the existence of additional mechanisms that render p53 nonfunctional in melanoma.

Activation of oncogenes can be accomplished by activated mutations, including *Ras* and *RAF* family members. Activating mutations of *Ras* in melanoma have been observed at an incidence of 10-15 % (Chin, 2003). *NRAS* and *HRAS* are the two common types of mutations, which are proposed to play important roles in the RAS-signaling pathway in melanoma genesis. *BRAF* activation is one of the most frequent melanoma-associated genetic events, since approximately 60 % of melanomas was found to carry the *BRAFV599E* mutation (Davies, 2002). The *BRAF* mutations were found in both benign and malignant melanocytic lesions, which suggest a key role of *BRAF* in transformation and melanoma development (Chin, 2003).

Therapeutic resistance is another hallmark of malignant melanoma, since melanoma tumors are strongly refractory to both chemo- and radio-therapy. One strategy that is currently being tried to treat melanoma patients is gene transfer delivery to certain melanoma of an adenoviral vector expressing wt p53 (Sotomayor et al., 2002). However, it is unclear if this or other current efforts to find a cure for melanoma will be successful.

2.5.3 **Integrin αvβ3 in melanoma progression**

Expression of integrin αvβ3 has been linked to malignant melanoma progression. For example, the vertical growth phase of malignant melanoma displays high expression levels of integrin αvβ3 as compared to horizontally growing melanoma in the epidermis (Albelda et al., 1990; Seftor et al., 1999; Van Belle et al., 1999). Overexpression of integrin β3 through *in vivo* gene delivery of integrin β3 gene promoted invasive melanoma growth from the epidermis into the dermis in three-dimensional (3D) skin reconstructs (Hsu et al., 1998). Integrin αv was also shown to promote melanoma metastatic potential (Felding-Habermann et al., 1992). Moreover, integrin αvβ3 promoted melanoma cell survival as shown in a 3D-collagen gel model *in vitro*, and in full thickness human skin *in vivo* (Montgomery et al., 1994; Petitclerc et al., 1999). In addition, block of integrin αvβ3 by an antagonistic anti-integrin αvβ3 mab induced melanoma cell apoptosis and thereby prevented melanoma tumor growth in mice. Furthermore, re-constitution of the integrin αv subunit into αv-negative melanoma cells rescued cell survival in 3D-collagen as well as in human skin and restored melanoma tumor growth *in vivo* (Montgomery et al., 1994; Petitclerc et al., 1999). Recently, integrin αvβ3 was also found to bind to the endothelial cell adhesion molecular L1, and this adhesive interaction might promote migration of melanoma cells across the endothelium (Voura et al., 2001).

The main part of my thesis was to investigate integrin αv-mediated melanoma cell survival mechanism.

2.6 THE TUMOR SUPPRESSOR p53

The p53 protein is a *bona fide* tumor suppressor. Upon various types of stress, including oncogenic stimuli and DNA damage, p53 is activated and triggers diverse cellular processes, including cell growth arrest and apoptosis. The importance of p53 for tumor suppression is underscored by the fact

that defects of p53 function occur in almost all human cancers. In approximately half of human cancers, p53 is inactivated directly as a result of mutations within the *TPp53* gene (Hollstein et al., 1999). Some cancers, although harbouring wild-type (wt) p53, are defective in the pathway of p53-activated cell cycle arrest or apoptosis due to viral infection, mouse double minute-1 (MDM2) overexpression, ARF or ATM deficiency (Wang et al., 2003). In melanoma, p53 is rarely mutated, but its function is somehow impaired (Satyamoorthy et al., 2001). p53 is also known as the 'guardian of the genome', since inactivation of p53 and p53 pathways accelerate genomic instability (Vogelstein et al., 2000). Loss of p53 functions promotes tumor progression, resistance to treatment and constitutes poor prognosis (Kirsch and Kastan, 1998).

Below, I will discuss certain aspects of p53 function and its regulatory mechanisms that are closely correlated with my present study.

2.6.1 **Apoptotic activities of p53**

Activation of apoptosis is an important function of the tumor suppressor p53. Numerous studies suggest that p53 triggers apoptosis through transcription-dependent and –independent mechanisms (Vousden and Lu, 2002), which contributes to the activation of the intrinsic mitochondrial as well as the extrinsic death receptor apoptotic pathways (discussed in section 2.4.4).

Transcriptional-dependent apoptosis

p53 serves as a tetrameric transcription factor through binding to specific DNA sequences (Ko and Prives, 1996) and transcriptionally controls apoptosis (Fridman and Lowe, 2003). p53 transcriptionally activates many proapoptotic Bcl-2 family members, including Bax, Bid, Noxa and PUMA (Miyashita et al., 1994; Nakano and Vousden, 2001; Oda et al., 2000a; Sax et al., 2002). These proteins are involved in induction of the intrinsic mitochondrial apoptotic pathway. p53 transactivates other apoptotic effectors, including Apaf1 (Soengas et al., 1999), which acts as a co-activator of caspase 9 and mediates activation of downstream caspase cascades leading to apoptosis. In addition, p53 also transrepresses many antiapoptotic genes, including Bcl-2, Bcl-XL and Survivin (Johnstone et al., 2002).

The death receptors FAS and PIDD (p53-induced protein with a death domain) are identified as the direct transcriptional targets of p53, these receptors are known to mediate an extrinsic apoptotic pathway (Lin et al., 2000; Maecker et al., 2000; Owen-Schaub et al., 1995). p53 may also activate apoptosis through engaging caspase 8 (Ding et al., 2000).

Moreover, p53 can also counteract survival signaling by its ability to transactivate PTEN, a negative regulator of PI-3K survival pathway (Fridman and Lowe, 2003).

Transcriptional-independent apoptosis

In the absence of transcription, p53 can elicit Bax-dependent apoptosis through translocation of Bax to mitochondria, leading to an increase in mitochondrial membrane permeability and subsequent release of cytochrome c (Chipuk et al., 2004; Chipuk et al., 2003). In addition, p53 can also directly translocate to mitochondria and mediate mitochondrial apoptosis (Mihara 2003). More recently, p53 was found to interact with the mitochondrial membrane protein Bak and form a p53-Bak complex. This complex can disrupt the interaction with Bak of the anti-apoptotic Bcl-2 member Mcl1, leading to a release of cytochrome c from mitochondria and subsequent activation of apoptosis (Leu et al., 2004).

Figure 5 summarizes p53-mediated apoptotic pathways.

2.6.2 **p53 post-translational modifications**

The tumor suppressor p53 is a tightly regulated protein. Several post-translational modifications are involved in the regulation of its stabilization, accumulation in the nucleus and transcriptional activation. These modifications include ubiquitination, sumoylation, neddylation, phosphorylation and acetylation (Bode and Dong, 2004; Harper, 2004). I will focus on discussing p53 ubiquitination, phosphorylation and acetylation.

Ubiquitin-mediated proteasome degradation is involved in regulation of p53. In unstressed cells, p53 is maintained at low levels through p53 interacting with MDM2, an ubiquitin ligase (Barak et al., 1993; Haupt et al., 1997). Because MDM2 is a transcriptional target of p53, MDM2-meditated proteasomal degradation of p53 constitutes a negative feedback mechanism (Oren, 1999; Wu et al., 1993). In addition, MDM2-indenpendent mechanisms, such as JNK stimulation, can also promote degradation of p53 through the ubiquitin-proteasome system (Fuchs et al., 1998).

Phosphorylation and acetylation are two common post-translational modifications in the regulation of p53 (Brooks and Gu, 2003). So far, 17 p53 phosphorylation sites have been described in human cells upon DNA damage induced by ionizing radiation or ultraviolet-light irradiation (Bode and Dong, 2004). Among these, Ser 46 phosphorylation appears to be importantly involved in p53 apoptotic function upon UV stimuli (Oda et al., 2000b). Acetylations of p53 by the cofactor p300/CBP, a histone acetyl transferase, occur at multiple lysine residues in the carboxy-terminal regulatory domain. Of these, acetylation of Lys382 has been found to be involved in p53-mediated apoptosis. For example, a recent study showed that p300-mediated acetylation of Lys382 controls p53-dependent apoptosis upon DNA damage through regulation of PUMA/p21 expression (Iyer et al., 2004).

2.6.3 **Regulation of p53 conformation**

p53 has a flexible protein conformation. To this end, two distinct conformations have been identified in cells by use of conformation-specific monoclonal antibodies (Bell et al., 2002). The monoclonal antibodies PAb1620 and PAb246 are specific for a folded, active conformation of p53 (Cook and Milner, 1990). Several other antibodies, including PAb240, recognize an unfolded, inactive conformation of p53 and can bind to unfolded p53 proteins (Gannon et al., 1990; Legros et al., 1994; Vojtesek et al., 1995).

The p53 activity strictly depends on its active, folded conformation (Bullock and Fersht, 2001). Point mutations (structural mutations) often cause a shift of the folded, active p53 conformation to an unfolded, inactive form, leading to p53 inactivation. For example, p53R175H, one of the most common structural mutations within *TPp53* gene, preferentially displays an unfolded, inactive PAb240 conformation. The structure mutation V143A displays a temperature-induced mutant conformation; at 32°C, it is recognized by PAb1620 and at 37°C by PAb240. Therefore, mutation-induced unfolding may explain p53 inactivation in more than half of human cancers. However, it is unclear whether the p53 conformation may affect the activity of wt p53 in tumor cells.

Several attempts are initiated to restore mutant p53 function as a new strategy for the development of tumor therapy. The restoration of an active conformation of mutant p53 originally derives from application of antibodies and peptides that could specifically bind to the p53 carboxy-terminus and promote p53 DNA-binding affinity (Abarzua et al., 1995; Selivanova et al., 1997). Rational designs then led to the identification of a short peptide, CDB3, with similar p53-activating properties (Friedler et al., 2002). Recently, a pharmacological rescue of mutant p53 conformation and function by a small compound CP31398 was achieved through stabilizing the active conformation of the p53-DNA binding domain (Foster et al., 1999). Furthermore, using a cell-based random screen of a chemical

library, PRIMA-1 (**p**53 **R**eactivation and **I**nduction of **M**assive **A**poptosis) was identified as capable of converting an unfolded conformation of mutant p53 to an active folded form, inducing apoptosis in a mutant p53-dependent manner (Bykov et al., 2002). PRIMA-1^{MET}, an analogue of PRIMA-1 with increase activity (Bykov et al., 2005), was applied in part of my present study to investigate whether also the conformation of wt p53 could be manipulated pharmacologically. Another recent approach to activate p53 was achieved through stabilizing wt p53 by blocking its interaction with MDM2. This led to identification of Nutlin (Vassilev et al., 2004), and the small organic compound RITA (Issaeva et al., 2004). For example, RITA was found to induce a conformational change that blocks interaction of p53 with its negative regulators, including human homology of MDM2 (HDM-2), which targets p53 for proteasomal degradation. These strategies of p53-targeting may provide novel promising approaches that could contribute to the development of tumor therapy.

In section 2.5.2, I mentioned that p53 is rarely mutated in melanoma (Castresana et al., 1993; Lubbe et al., 1994; Montano et al., 1994). However, melanoma tumors are not sensitive to ionizing radiation or chemotherapy (Jenrette, 1996; Satyamoorthy et al., 2000), implicating that the p53-controlled functions may be disrupted. An impaired function of p53 is often found to be associated with defects in the signaling pathways that are upstream or downstream of p53 (Satyamoorthy et al., 2000; Soengas et al., 2001; Vousden and Lu, 2002), but an impaired p53 function may be caused by the p53 protein itself. In fact, a modulation of the p53 conformation was suggested to associate with the regulation of embryonic stem cell differentiation (Sabapathy et al., 1997). The inactivation of p53 by a conformational change could allow the differentiating cells to escape from apoptosis. The present study addressed the effects of p53 conformation on p53 activation and function in malignant melanoma.

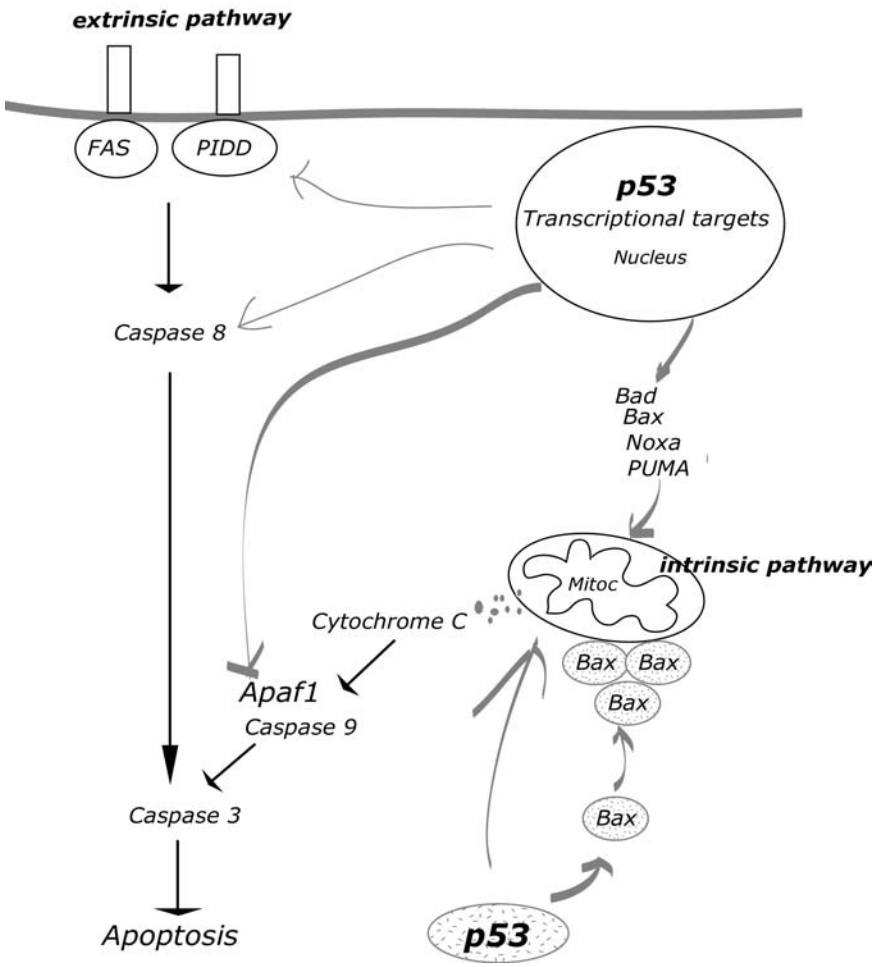

Figure 5 Schematic drawing of suggested p53-mediated apoptotic pathways related to the present investigation. p53 transcriptionally transactivates numeous downstream targets including Apaf1, Bad, Bax, Noxa and PUMA known to mediate an intrinsic mitochondrial apoptosis. p53 also directly translocates to mitochondria or targets to mitochondria through interactions with apoptotic proteins involving Bax in a transcription-independent manner. In addition, p53 regulates an extrinsic apoptotic pathway through transcriptional regulation of its downstream targets death receptors FAS and PIDD, and caspase 8.

2.7 THREE-DIMENSIONAL CELL CULTURE MODELS

2.7.1 **Characteristics of 3D cell culture environment**

In living organisms, tissue cells are growing in a 3D-microenvironment where they are communicating with one and another and interacting with their surrounding ECM. Therefore, in order to more closely reflect the situation *in vivo*, a 3D-culture environment may represent a useful model for researchers in cell biology. In contrast to conventional, regular 2D-cultures where artificial substrates such as plastic are probably distorting the cells by enforcing them to adjust to flat and rigid surface (Elsdale and Bard, 1972), the 3D-culture provides a more authentic environment. In fact, molecular composition of the 3D-ECM environment may facilitate to most cell proliferate and survival as they live inside the organisms (Cukierman et al., 2002).

Many studies have demonstrated that cells cultured in 3D generate different signaling and display different behavior as compared to those observed in 2D. For example, through its interaction with integrins, 3D-matrix can induce special 3D-matrix adhesion structures, called 3D-adhesions (Cukierman et al., 2002). They were elongated and spindle shape, and clearly morphologically different from the focal and fibrillar adhesions characterized on 2D-substrates (Zamir et al., 1999) (discussed in section 2.2.2). 3D-fibronectin-mediated adhesions are characterized with low levels of phosphorylated FAK, although the FAK protein is abundant. This differs from the highly phosphorylated FAK found in focal adhesions, implicating that 3D-ECM may transduce their specific signaling pathways (Cukierman et al., 2002). EGF-receptor signaling were also found to be different in response to cell adhesion within 3D-matrices as compared to attachment onto 2D-tissue culture plates (Cukierman et al., 2001; Wang et al., 1998). The differences in cell polarity and interaction with the surrounding ECM within 3D-culture might also dictate the susceptibility of normal and cancer cells to the cancer chemotherapy in terms of induction of apoptosis (Weaver et al., 2002). In a 3D-collagen model, the expression of matrix metalloproteinase 13 (MMP13) was upregulated through integrin $\alpha 1\beta 1$ and $\alpha 2\beta 1$-mediated activation of ERK, JNK and p38 MAPK (Ravanti et al., 1999). Importantly, in a 3D-collagen model, integrin αv was found to control melanoma cell survival (Montgomery et al., 1994), consistent with later findings in vivo (Petitclerk., 1999). The latter 3D-collagen model was applied in parts of my present study.

2.7.2 **Examples of 3D cell culture models**

3D reconstituted basement membrane culture

This model was developed in Mina Bissell's lab to mimic a microenvironment for mammary cells. This model has been used to study mammary breast carcinoma morphology, polarity, growth, survival and signaling, as well as application for the study of invasive, tumorigenicity in nude mice (Weaver et al., 1995).

3D cell-derived fibronectin matrix

3D-matrix derived from culture cells that contains fibronectin has been extensively used for growing cells under quasi-physiological conditions, yet its capacity to support specific molecular types of adhesions has not been determined (Vlodavsky, 1999; Vlodavsky et al., 1982).

3D-collagen gel

This model was first described by Elsdale and Bard (Elsdale and Bard, 1972) in 1972, containing dermal collagen type I that polymerize to form a 3D-fibrous network mimicking a dermal *in vivo*

environment for cell growth, where approximately 90 % of the protein is collagen type I. In the 3D-collagen, the morphological changes of fibroblasts were partially similar to those *in vivo*. The 3D-collagen gels have also been used to study cell motility (Friedl and Brocker, 2000), melanoma cell survival (Montgomery et al., 1994) and primary melanoma explants (Hegerfeldt et al., 2002).

Peptide Hydrogel (BD™PuraMatrix™)

The Peptide Hydrogel is a newly established model based on a scaffold formed by self-assembling peptides that comprise standard amino acid (1 % w/v) and 99 % water. Under physiological conditions, the peptide component self-assembles into a 3D hydrogel that exists in a nanometer scale fibrous structure with just the right porosity to slowly distribute nutrients and other necessary biological molecules to embedded cells, resulting in a realistic 3D-microenvironment. This model has been used to study cell differentiation, attachment, proliferation, invasion, and *in vivo* analyses of tissue regeneration (Semino et al., 2004; Semino et al., 2003; Zhang et al., 1995a).

3 AIMS OF THE PRESENT INVESTIGATION

Integrins function as transmembrane adhesion receptors that mediate cell interactions with their surrounding ECM. Upon ligation, integrins transduce numerous signaling pathways, leading to control of various cellular functions, including cell proliferation and survival. The overall aim of the present study was to investigate the role of integrin signaling in the control of cell proliferation and survival. The specific goals for each study in this thesis were:

I. To develop a method that specifically analyzes integrin signaling without contribution of cell spreading

II. To elucidate mechanisms of integrin signaling in control of Cdk2-inhibitors $p21^{CIP1}$ and $p27^{KIP1}$

III. To clarify mechanisms of integrin αv-mediated melanoma cell survival

IV. To assess mechanisms of integrin αv-mediated inactivation of tumor suppressor p53

4 RESULTS AND DISCUSSION

4.1 An immobilized anti-integrin monoclonal antibody developed to examine specific integrin signaling independent of cell spreading (paper I)

Integrin signaling-mediated responses are commonly studied by allowing cell attachment to ECM proteins coated onto a solid surface. Upon ligation, integrin signaling is transduced into the cell, but parts of the signaling responses also stem from cell spreading (Chen et al., 1997). To analyze specific integrin-mediated responses independent of cell spreading, we developed a method engaging integrin signaling by use of an immobilized mab (JBS5) directed against the fibronectin receptor integrin $\alpha5\beta1$. In this case, this antibody acts as an agonistic integrin-ligating protein and promotes integrin signaling. Using this method, we found that cells plated onto immobilized mab JBS5 did not spread, but displayed a strong reduction of the Cdks-inhibitors $p21^{CIP1}$ and $p27^{KIP1}$. Therefore, this method can be used to analyze specific integrin signaling independent of cell spreading.

4.2 Integrin ligation activates proteasomal degradation of the Cdk2-inhibitor $p21^{CIP1}$ (papers I and II)

The Cdk2-inhibitors $p21^{CIP1}$ and $p27^{KIP1}$ are negatively regulated by anchorage during cell proliferation (Fang et al., 1996; Strömblad et al., 1996; Zhu et al., 1996). To address the role of integrin signaling in the regulation of $p21^{CIP1}$ and $p27^{KIP1}$, three distinct cell lines were allowed to attach to different ECM proteins, including fibronectin and vitronectin. To analyze specific responses mediated by integrin signaling independent of cell spreading, an immobilized anti-integrin mab was used as described in paper I. To exclusively analyze the effect of integrin signaling, cells were exposed in the absence of serum to avoid any contribution from growth factor signaling. Our results showed that integrin-mediated cell attachment to ECM induced rapid downregulation of $p21^{CIP1}$ and $p27^{KIP1}$ but not integrin-independent attachment to poly-L-lysine. Surprisingly, we found that integrin ligation could activate proteasomal proteolysis, leading to degradation of $p21^{CIP1}$ and $p27^{KIP1}$. Our results revealed the first example of integrin signaling capable of controlling protein stability. Moreover, we showed that ubiquitination was not required for integrin-mediated proteasomal degradation of $p21^{CIP1}$. This was an unexpected finding, but several other reports have also described mechanisms of an ubiquitin-independent degradation of $p21^{CIP1}$. For example, direct ubiquitination of $p21^{CIP1}$ was not necessary for its turnover by the proteasome (Sheaff et al., 2000). A recent report also supported our finding that disruption of essential components for the ubiquitination mechanism did not influence endogenous $p21^{CIP1}$ degradation (Chen et al., 2004). Moreover, $p21^{CIP1}$ was suggested to directly bind to the C8 α-subunit of the 20S proteasome complex leading to degradation of $p21^{CIP1}$ (Touitou et al., 2001). Therefore, integrin-induced proteolysis of $p21^{CIP1}$ may represent a physiological proteasomal pathway that is independent of ubiquitination.

4.3 Integrin-activated Cdc42/Rac1 signaling pathway controls proteasomal degradation of $p21^{CIP1}$ (paper II)

Integrin-activated signaling pathways, including through FAK, PI-3K and ERK1/2, are critically involved in the control of cell proliferation (Giancotti and Ruoslahti, 1999). The small GTPases Cdc42, Rac1, and Rho have been indicated to be involved in the control of cell cycle progression through the G-1 phase, including control of cyclin D1 expression (Coleman et al., 2004). However, it was unclear if Cdc42 or Rac signaling induced by integrins may affect Cdk2 inhibitors. Using a GST-PAK1 pull down assay, we showed that integrin ligation could activate a Cdc42 to Rac1 signaling pathway. Importantly, we found that integrin-activated Cdc42 to Rac signaling could activate

proteasomal degradation of $p21^{CIP1}$. However, block of signaling through FAK, MEK1/ERK1/2 or PI-3K did not revert the integrin-induced down-regulation of $p21^{CIP1}$ (Bao and Strömblad, unpublished results). Instead, we link the integrin-mediated down-regulation of $p21^{CIP1}$ to signaling by the small GTPases Cdc42 and Rac. Ras signaling has been shown to stimulate $p27^{KIP1}$ degradation and to regulate $p21^{CIP1}$ expression in response to growth factors (Hirai et al., 1997; Olson et al., 1998; Takuwa et al., 1999). Our finding that integrin activation of endogenous Cdc42/Rac1 signaling induces proteasomal degradation of the Cdk2-inhibitor $p21^{CIP1}$ might contribute to clarify the complex function of small GTPases in cell cycle progression.

Upon integrin ligation, the mechanism involved in cdc42-mediated subsequent activation of Rac1 is unclear. PIX, a Cdc42/Rac GEF has been suggested to link Cdc42 to Rac1 activation by coupling of the cdc42 effector PAK1 (Manser et al., 1998). However, it remains to be elucidated whether these exchange factors might be involved in integrin-mediated Cdc42 to Rac1 activation.

Integrin-activated Cdc42 and Rac1 are involved in the regulation cell spreading (Clark et al., 1998; Price et al., 1998). One may suspect that Cdc42/Rac1 promotes $p21^{CIP1}$ degradation through affecting cell spreading, since $p27^{KIP1}$ degradation was shown to be regulated by cell spreading (Huang et al., 1998). However, our results showed that suppression of Cdc42 or Rac1 did not block activation of FAK or ERK1/2 signaling induced by cell attachment, indicating the existence of integrin specific signaling. In addition, our results also showed that down-regulation of $p21^{CIP1}$ and $p27^{KIP1}$ could occur in the absence of cell spreading, when cells were attached onto immobilized mab JBS5 (paper I). Thereby we concluded that an integrin-induced signaling pathway through Cdc42/Rac1 specifically causes proteasomal proteolysis of $p21^{CIP1}$.

4.4 Integrin αv promotes melanoma cell survival by inactivating p53 (paper III)

Integrin αv has been found to be critical for melanoma cell survival *in vitro* and *in vivo* models (Montgomery et al., 1994; Petitclerc, et al 1999). In this study, we examined the mechanism of integrin αv-mediated melanoma cell survival by using a 3D-collagen model mimicking the pathophysiological environment of malignant melanoma growth in dermis *in vivo*. We cultured integrin αv-positive M21 and M0-αv cells, and corresponding αv-negative M21L and M0 cells in 3D-collagen. We firstly confirmed previous findings that integrin αv is required for melanoma cell survival in 3D-collagen (Montgomery et al., 1994). Importantly, we found that integrin αv suppressed p53 DNA-binding activity in 3D-collagen. Furthermore, we showed that suppression of p53 by dominant negative p53 or p53-siRNA obviated the need for integrin αv for melanoma cell survival in 3D-collagen and for melanoma tumor growth *in vivo*. This suggests that integrin-mediated inactivation of p53 functionally controls melanoma cell survival.

In addition, we investigated whether post-translational modifications by phosphorylation and acetylation may affect p53 activity. Our results showed that p53-K382-acetylation was downregulated in integrin αv-positive M21 and M0-αv cells, associated with decreased p53 activity upon integrin αv ligation, correlating with a downregulation of p300, a cofactor for p53 acetylation. Integrin αv control of p300 may therefore play a role for p53-acetylation at K382.

We also examined the p53 transcriptional downstream targets Apaf1, Bax, Bcl-2 and PUMA, all are known to be involved in apoptosis regulation. We found that PUMA levels were markedly increased in integrin αv-negative M21L and M0 cells as compared to M21 and M0-αv cells. Our results also indicated that PUMA might be a downstream target of p53 (Jeffers et al., 2003).

It has been suggested that unligated integrin αvβ3 may induce epithelial cell apoptosis in 3D-collagen by recruitment and activation of caspase-8 (Stupack et al., 2001). However, we found that loss of integrin αv activated caspase 9 but not caspase 8, suggesting that loss of integrin αv may activate a mitochondrial apoptosis pathway since caspase 9 is essential for p53-induced apoptosis (Soengas et al., 1999).

4.5 Integrin αv-mediated p53 inactivation controls a MEK1-dependent melanoma cell survival pathway (paper III)

Integrin-activated Raf/MEK/ERK1/2 signaling has been implicated in the control of cell survival (Howe et al., 2002), and anchorage is required for activating melanocyte ERK1/2 signaling (Conner et al., 2003). Although most melanoma cells in regular 2D-culture display an active ERK1/2 because of activated BRAF V599E mutation (Satyamoorthy et al., 2003), our results showed that activation of MEK1/ERK1/2 signaling depended on integrin αv within 3D-collagen. Importantly, this integrin-activated signaling pathway was necessary for melanoma cell survival in 3D-collagen because block of MEK1 activity by specific inhibitor induced apoptosis. These results are consistent with an *in vivo* need for integrin αvβ3 for sustained vascular cell ERK1/2 activation during angiogenesis (Eliceiri et al., 1998). Furthermore, integrin αvβ3 was shown to trigger FAK as well as PAK1-mediated c-Raf-S338 phosphorylation in angiogenic endothelial cells leading to activation of MEK1 and ERK1/2 (Hood et al., 2003). However, we found neither FAK-Y397 or c-Raf-S338 phosphorylation, nor PAK1 kinase activity to be regulated by integrin αv in melanoma cells in 3D-collagen (Bao et al, unpublished observations). However, surprisingly, we found that p53 acted upstream of MEK1 signaling and functionally controlled MEK1-mediated melanoma cell survival, since stable transfection with dnp53-His175 or p53-siRNA rescued integrin αv-negative M21L cell MEK1 and ERK1/2 activities in 3D-collagen.

4.6 Integrin αv induces an unfolded, inactive wt p53 conformation in melanoma cells (paper IV)

p53 mutations cause unfolded conformation leading to inactivation of p53 (Cook and Milner, 1990; Gannon et al., 1990). However, no published study has addressed if wt p53 may adopt an unfolded conformation that could cause p53 inactivation in melanoma cells. To investigate how integrin αv mediates p53 inactivation, we therefore analyzed wt p53 conformation in three distinct melanoma cell lines cultured in 3D-collagen by ELISA using the conformation-specific monoclonal antibodies (mab) PAb1620 and PAb240 to detect folded (active) and unfolded (inactive) conformations, respectively (Bykov et al., 2002). Firstly, we found that 3D-collagen could confer an inactive, unfolded wt p53 conformation (240 epitope) in all three melanoma cell lines. To examine the potential role of integrin αv in the modulation of wt p53 conformation, we compared p53 conformation in αv-positive M21 cells and a M21 subpopulation, M21L cells, that lacks integrin αv. Surprisingly, we found that integrin αv could enforce wt p53 into an inactive conformation within 3D-collagen. Importantly, the p53 conformation state was consistent with p53 activation and function, since integrin αv–positive M21 cells that displayed an inactive conformation and also had low p53 activity and survived within 3D-collagen, whereas αv–negative M21L cells that displayed an active conformation also displayed high p53 activity and p53-dependent apoptosis described in paper III. Therefore, our results suggest that the regulation of wt p53 conformation is critical for the integrin αv control of melanoma cell survival in a 3D-environment. These results for the first time demonstrate a controlled wt p53 conformational regulation *in lieu* of p53 mutations in tumor cells, which may contribute to explain the lack of need for p53 mutations in malignant melanoma.

4.7 PRIMA-1^{MET} reactivates wt p53 conformation and its apoptotic function in melanoma cells (paper IV)

PRIMA-1^{MET}, an analogue of PRIMA-1 with increased activity, can revert an inactive, unfolded conformation of mutant p53 protein to an active, folded form (Bykov et al., 2002; Bykov et al., 2005). Our results suggested that PRIMA-1^{MET} could also switch an unfolded, inactive wt p53 conformation to a folded, active form in three distinct melanoma cell lines within 3D-collagen. To examine if PRIMA-1^{MET} could also activate melanoma cell apoptosis in a p53-dependent manner, the p53 protein was knocked-down by stably transfecting p53-siRNA (Brummelkamp et al., 2002) in M21 cells. We showed that PRIMA-1 activated melanoma cell apoptosis in a wt p53-dependent manner, which supports a role for regulation of wt p53 conformation in the control of melanoma cell survival.

We also investigated PRIMA-1^{MET}-triggered apoptosis mechanisms by examining apoptotic proteins Apaf1 and PUMA levels in M21 and p53 knocked-down M21-p53siRNA cells. We indicated that PRIMA-1^{MET} could induce a p53-dependent induction of transcriptional target PUMA, and that PUMA could be acting downstream of p53 in activation of apoptosis. Given that p53 can induce apoptosis by a direct localization to mitochondria (Mihara et al., 2003), that PRIMA-1 activated mutant p53 and elicited bax-dependent apoptosis in a transcriptional-independent manner (Chipuk et al., 2003), and that PRIMA-1 promoted mutant p53 translocation to the cytoplasm (Bykov et al., 2002), PRIMA-1^{MET} may induce transcriptional-independent apoptosis in melanoma cells.

In addition, we found caspase 9 to be functionally involved in PRIMA-1^{MET}-induced apoptosis. This indicates that PRIMA-1^{MET} may activate a p53-dependent, mitochondrial apoptotic pathway.

4.8 PRIMA-1^{MET} suppresses melanoma xenograft tumor growth in mice in a wt p53-dependent manner (paper IV)

Based on the fact that PRIMA-1^{MET} could trigger melanoma cell apoptosis in 3D-collagen, we tested the effects of PRIMA-1^{MET} on melanoma tumor growth *in vivo*. We found that PRIMA-1^{MET} treatment suppressed M21 tumor growth carrying wt p53, but did not inhibit tumor growth of C8161 cells carrying a defective p53-truncation. This may suggest that PRIMA-1^{MET} suppresses melanoma tumor growth depending on a functional p53. In addition, PRIMA-1^{MET} also rendered no effect on tumor growth of M21-p53siRNA cells, strongly indicating that PRIMA-1^{MET} suppression of melanoma tumor growth is dependent of wt p53.

We also found that an intermittent regimen treatment of PRIMA-1^{MET} markedly inhibited FM88 melanoma tumor growth, indicating that PRIMA-1^{MET} efficiently inhibits melanoma tumor growth of two distinct origins. However, surprisingly, continuous treatment (everyday) with PRIMA-1^{MET}, was less efficient, for both FM88 and M21 cells (data not shown). This indicates that optimization of PRIMA-1^{MET}dosing and regimen is an important issue. Nevertheless, our results clearly demonstrate proof of principle for efficacy of a wt p53-targeting compound inhibiting melanoma tumor growth.

4.9 The 3D-collagen gel model for studying integrin signaling-mediated melanoma cell survival (papers III and IV)

Using a three-dimensional collagen model mimicking a pathophysiological environment of melanoma growth in the dermis *in vivo,* we investigated regulatory mechanisms for integrin αv-mediated melanoma cell survival. We found that wt p53 conformation, activity and apoptotic function were regulated by integrin αv only within 3D-collagen. Moreover, integrin-activated MEK1/ERK1/2

signaling was only observed in 3D as well as the control of MEK1 signaling by integrin αv-mediated p53 regulation. Furthermore, regulation of wt p53 conformation and function by PRIMA-1^{MET} was observed only in the 3D-collagen environment. Importantly, our results from the 3D-collagen environment were similar to our results on melanoma tumor growth *in vivo*: (1) Suppression of p53 activity rescued tumor growth of αv-integrin negative melanoma cells that otherwise did not grow. (2) PRIMA-1^{MET} induced melanoma cell apoptotic death in 3D-collagen and also markedly suppressed melanoma xenograft tumor growth in mice. In fact, several studies have demonstrated that integrin-mediated cell-matrix interactions trigger different signaling and cellular behavior in 3D-environment as compared with regular 2D-culture conditions (Cukierman et al., 2002; Jacks and Weinberg, 2002). Our results reveal another example how important a 3D-environment can be for studying integrin-mediated cellular responses since we would not have been able to find any of the presented mechanisms if the studies had been performed under 2D-conditions. Notably, the application of 3D-culture models to study integrin function may more closely reflect the situations in living organisms.

5 CONCLUDING REMARKS

The present investigation reveals several new effects of integrin signaling in the control of cell proliferation and survival.

In response to interaction with the ECM, we found that integrins control the key cell cycle regulators $p21^{CIP1}$ and $p27^{KIP1}$ by activating a proteasomal proteolysis mechanism. This for the first time reveals that integrins are capable of controlling protein stability. An integrin-activated signaling pathway involving cdc42 and Rac1 is critically involved in the control of this proteasomal degradation of $p21^{CIP1}$. A hypothetical model for integrin regulation of proteasomal proteolysis of $p21^{CIP1}$ is presented in Figure 6. Integrin-mediated proteasomal proteolysis might contribute to anchorage-dependent cell cycle control.

For the roles of integrins in control of melanoma cell survival, integrin αv displays a similar mechanism as it acts in angiogenesis, where integrin αv is capable of suppressing tumor suppressor p53 activity (Brooks et al., 1994; Eliceiri et al., 1998; Strömblad et al., 1996; Strömblad et al., 2002). Furthermore, integrin αv inactivated p53 by inducing an unfolded wt p53 conformation and promoted MEK1 signaling, demonstrating the first example of a cell adhesion receptor that can control a conformational regulation of a tumor suppressor p53 in tumor cells *in lieu* of mutations. Through reactivating wt p53 conformation, the small organic compound PRIMA-1^{MET} induced a marked p53 apoptotic function and suppressed melanoma xenograft tumor growth. This may provide a novel approach to the urgently needed development of melanoma therapy. Figure 7 illustrates a hypothetical model for the mechanism involved in integrin-αv-mediated melanoma cell survival.

Future studies would be necessary to pinpoint the remaining underlying molecular mechanisms that were not elucidated in the present study. For example, it would be interesting to address the signaling upstream of the small GTPases Cdc42 and Rac1 upon integrin ligation, and to address how small GTPases leads to proteasome degradation of $p21^{CIP1}$ through identifying more of the pathways involved. It would also be of exceptional interest to clarify how integrin αv regulates wt p53 conformation in terms of investigating molecules or cofactors involved in this conformational regulation of wt p53, which act downstream of integrins αv. Another future development that has already been initiated is to further develop new strategy for treatment of malignant melanoma, which could be useful in clinical trials in the future.

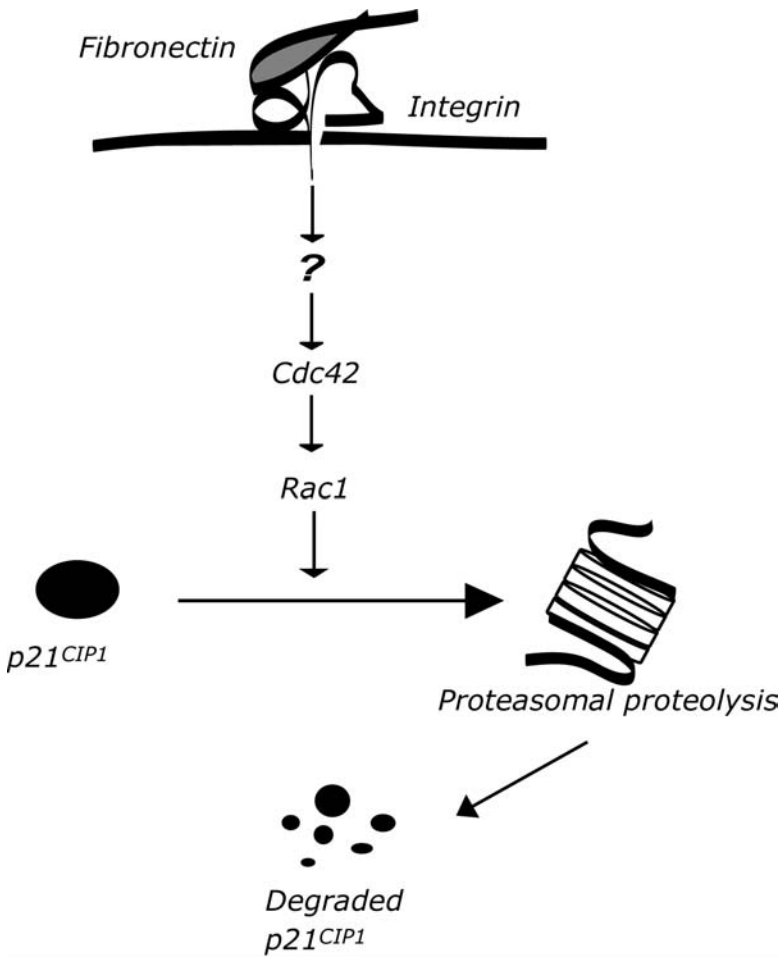

Figure 6 A hypothetical model for integrin regulation of proteasomal degradation of Cdk2-inhibitor $p21^{CIP1}$ through activating a Cdc42 to Rac1 signaling pathway. Integrin ligation to fibronectin leads to activation of a Cdc42 to Rac1 signaling pathway that promotes proteasomal proteolysis of $p21^{CIP1}$.

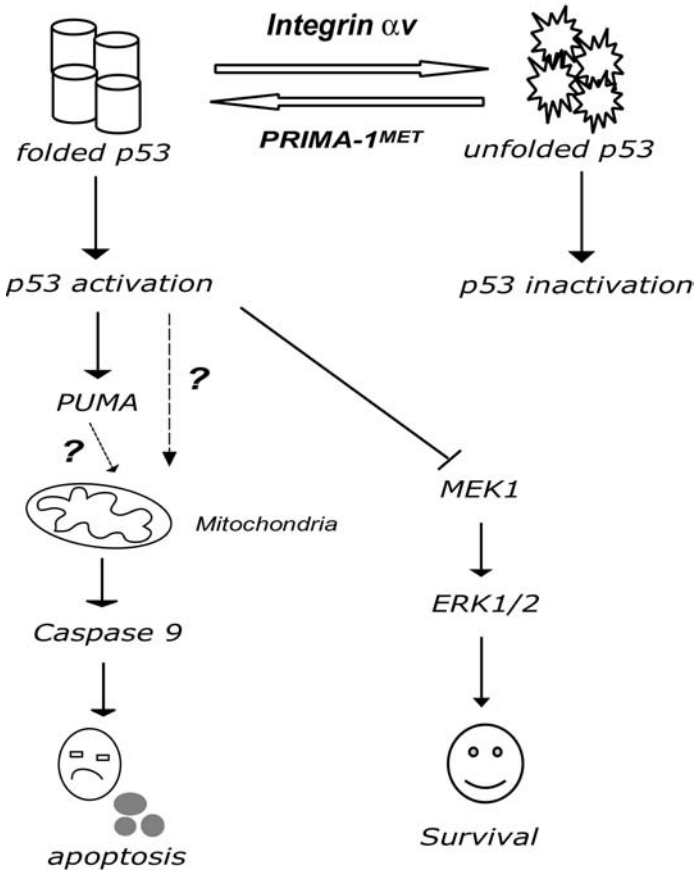

Figure 7 A hyothetical model for how integrin αv may control melanoma cell survival. Expression of integrin αv promotes p53 inactivation in 3D environment by inducing an unfolded p53 conformation. Lack of integrin αv leads to upregulation of apoptotic protein PUMA, and enhancement of cleaved caspase 9, suggesting a mitochondrial apoptotic pathway. Lack of integrin αv also leads to p53-dependent inhibition of a MEK1-ERK1/2-mediated melanoma cell survival pathway. PRIMA-1^{MET} switches an unfolded p53 conformation to a folded form, leading to activation of p53 and melanoma cell apoptosis.

6 ACKNOWLEDGEMENTS

I would like to thank the Department of Laboratory Medicine, Karolinska Institute for providing an excellent environment, allowing me to complete my doctoral study. The experience shared here would be an unforgettable part of my career. I would also like to express my greatest gratitude to the people who ever contribute to my thesis in one way or another. In particular, I would also like to mention:

My advisor, ***Staffan Strömblad***, for your excellent supervision and creating an ambitious scientific atmosphere. You always provide opportunities for me, either to discuss with visiting scientists worldwide or to attend international conferences to catch knowledge on the frontier of cell biology. I quite enjoy many inspiring discussion that we have had. I am quite grateful to your patience for correcting my manuscripts because you always try to dig out all my potentials to write them in the best way. One can not forget your delicious homemade cakes for the birthday celebration.

Galina Selivanova, for your kind help for teaching me a method, called EMSA, at my difficult time at the beginning of my study. With your help, the puzzling over my head for a half-year was suddenly swept away. I am also grateful to sharing your knowledge on p53 and all your suggestion for my studies.

Klas Wiman, for sharing your knowledge on p53, and nice collaborations with you. I am quite happy for sharing that pleasure time and having nice discussion at Dunedin p53 conference in New Zealand.

My dear friends in our group: ***Minna Thullberg,*** for your enthusiasm in helping with correction and giving valuable suggestion for my thesis, and for having nice discussion on my project. Thank you for your understanding, often providing various kinds of useful information, and often explaining music broadcasted from the radio. ***Annica Gad,*** especially for sharing those unforgettable days when we were in a small group at the beginning. We helped and discussed each other's project and learned each other's language etc. ***HongQuan Zhang,*** for sharing your knowledge on biochemistry, having a nice collaboration and ever having had nice chat on science. ***Helen Olofson,*** for sharing the same pipette for many years, and for your excellent assistance. Your sweet smiling makes me feel very comfortable. ***Zhilun Li***, ***Ghasem Nurani*** and ***Xu Xhao,*** for sharing the same lab and office, and being glad to work with you.

Göran Andersson, the head of Division of Pathology, for your support, understanding and generosity. ***Maj-Len Holm,*** for your excellent secretary works whenever I go to you.

Natalia Issaeva and ***Vladimir Bykov,*** for your excellent collaboration or help. ***Pernilla Lång,*** for your enthusiasm and computer support.

I would like to thank all my old friends who are living in other countries: ***Gensheng Wang,*** for numerous help from you and your wife ***Xing Liu***. ***Ping Huang/Wenxin Hunag,*** for understanding me, helping my family and sharing your delicious Guang-Dong meal during the period that we live at the same building. ***Guangxian Zhang/Liya Yu,*** for your help in many situations. ***Hong Zhu,*** for being my hair-dresser. ***Shimei Zhuang,*** for sharing sweet time with your family in Linköping. ***Yukun Li/Yi Yang*** and ***YongQing Guo*** for your hospitably entertainment in Beijing.

I would like to thank all the friends at the present ***badminton club*** for your friendship. Particularly, the ladys ***Chuyang Zhao***, ***Fang Zong***, ***Xiao Wang and Yingchun Dou,*** besides playing badminton, I have been enjoying to chat with all of you, with many topics, cooking, film, novel, travel...
I wish all of you best of luck.

My friends ***Hong Fang*** and ***Yunling Wang,*** for exchanging video cassettes and VCD, and/or exchanging knowledge for planting flowers, which make my life more colourful.

I also would like to express my greatest attitudes to my friends in China, ***Lihua Wang***, ***Yongyi jiang***, ***Xiaohong Cui*** and ***Jun Deng***, for your friendship and support.

I thank all my family members for help and support me during the period of my stay in Sweden. In particular, ***our parents*** for your invaluable support and understanding. My brother ***Wenhao,*** for your understanding and taking care of our parents.

Ming, my dear husband, thank you for your love, understanding, support …
Bilin, our lovely son, thank you for your intelligence and love, being the source of our joy.

The thesis is reproduced from: (1) the Journal of Cell Biology, 2004, Vol 167., 745-756. by the copyright permission of The Rockefeller University Press. (2) Molecular and Cellular Biology, 2002, Vol 22., 4587-4597. by the copyright permission of The American Society for Microbiology.

Stockholm, May 2005

7 REFERENCES

Abarzua, P., J.E. LoSardo, M.L. Gubler, and A. Neri. 1995. Microinjection of monoclonal antibody PAb421 into human SW480 colorectal carcinoma cells restores the transcription activation function to mutant p53. *Cancer Res*. 55:3490-4.

Albelda, S.M., S.A. Mette, D.E. Elder, R. Stewart, L. Damjanovich, M. Herlyn, and C.A. Buck. 1990. Integrin distribution in malignant melanoma: association of the beta 3 subunit with tumor progression. *Cancer Res*. 50:6757-64.

Allen, W.E., D. Zicha, A.J. Ridley, and G.E. Jones. 1998. A role for Cdc42 in macrophage chemotaxis. *J Cell Biol*. 141:1147-57.

Aplin, A.E., A. Howe, S.K. Alahari, and R.L. Juliano. 1998. Signal transduction and signal modulation by cell adhesion receptors: the role of integrins, cadherins, immunoglobulin-cell adhesion molecules, and selectins. *Pharmacol Rev*. 50:197-263.

Aplin, A.E., S.A. Stewart, R.K. Assoian, and R.L. Juliano. 2001. Integrin-mediated adhesion regulates ERK nuclear translocation and phosphorylation of Elk-1. *J Cell Biol*. 153:273-82.

Arnaout, M.A. 2002. Integrin structure: new twists and turns in dynamic cell adhesion. *Immunol Rev*. 186:125-40.

Ashkenazi, A., and V.M. Dixit. 1998. Death receptors: signaling and modulation. *Science*. 281:1305-8.

Bagrodia, S., and R.A. Cerione. 1999. Pak to the future. *Trends Cell Biol*. 9:350-5.

Barak, Y., T. Juven, R. Haffner, and M. Oren. 1993. mdm2 expression is induced by wild type p53 activity. *Embo J*. 12:461-8.

Bar-Sagi, D., and A. Hall. 2000. Ras and Rho GTPases: a family reunion. *Cell*. 103:227-38.

Bell, S., S. Hansen, and J. Buchner. 2002. Refolding and structural characterization of the human p53 tumor suppressor protein. *Biophys Chem*. 96:243-57.

Bissell, M.J., and D. Radisky. 2001. Putting tumours in context. *Nat Rev Cancer*. 1:46-54.

Blagosklonny, M.V., G.S. Wu, S. Omura, and W.S. el-Deiry. 1996. Proteasome-dependent regulation of p21WAF1/CIP1 expression. *Biochem Biophys Res Commun*. 227:564-9.

Bode, A.M., and Z. Dong. 2004. Post-translational modification of p53 in tumorigenesis. *Nat Rev Cancer*. 4:793-805.

Borradori, L., and A. Sonnenberg. 1999. Structure and function of hemidesmosomes: more than simple adhesion complexes. *J Invest Dermatol*. 112:411-8.

Boyer, S.N., D.E. Wazer, and V. Band. 1996. E7 protein of human papilloma virus-16 induces degradation of retinoblastoma protein through the ubiquitin-proteasome pathway. *Cancer Res*. 56:4620-4.

Brooks, C.L., and W. Gu. 2003. Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation. *Curr Opin Cell Biol*. 15:164-71.

Brooks, P.C., A.M. Montgomery, M. Rosenfeld, R.A. Reisfeld, T. Hu, G. Klier, and D.A. Cheresh. 1994. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell*. 79:1157-64.

Brugnera, E., L. Haney, C. Grimsley, M. Lu, S.F. Walk, A.C. Tosello-Trampont, I.G. Macara, H. Madhani, G.R. Fink, and K.S. Ravichandran. 2002. Unconventional Rac-GEF activity is mediated through the Dock180-ELMO complex. *Nat Cell Biol*. 4:574-82.

Brummelkamp, T.R., R. Bernards, and R. Agami. 2002. A system for stable expression of short interfering RNAs in mammalian cells. *Science*. 296:550-3.

Bullock, A.N., and A.R. Fersht. 2001. Rescuing the function of mutant p53. *Nat Rev Cancer*. 1:68-76.

Burridge, K., and K. Wennerberg. 2004. Rho and Rac take center stage. *Cell*. 116:167-79.

Bykov, V.J., N. Issaeva, A. Shilov, M. Hultcrantz, E. Pugacheva, P. Chumakov, J. Bergman, K.G. Wiman, and G. Selivanova. 2002. Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound. *Nat Med*. 8:282-8.

Bykov, V.J., N. Zache, H. Stridh, J. Westman, J. Bergman, G. Selivanova, and K.G. Wiman. 2005. PRIMA-1(MET) synergizes with cisplatin to induce tumor cell apoptosis. *Oncogene*.

Calalb, M.B., T.R. Polte, and S.K. Hanks. 1995. Tyrosine phosphorylation of focal adhesion kinase at sites in the catalytic domain regulates kinase activity: a role for Src family kinases. *Mol Cell Biol*. 15:954-63.

Castresana, J.S., M.P. Rubio, J.J. Vazquez, M. Idoate, A.J. Sober, B.R. Seizinger, and R.L. Barnhill. 1993. Lack of allelic deletion and point mutation as mechanisms of p53 activation in human malignant melanoma. *Int J Cancer*. 55:562-5.

Cayrol, C., and B. Ducommun. 1998. Interaction with cyclin-dependent kinases and PCNA modulates proteasome-dependent degradation of p21. *Oncogene*. 17:2437-44.

Chen, C.S., M. Mrksich, S. Huang, G.M. Whitesides, and D.E. Ingber. 1997. Geometric control of cell life and death. *Science*. 276:1425-8.

Chen, H.C., and J.L. Guan. 1994. Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase. *Proc Natl Acad Sci U S A*. 91:10148-52.

Chen, Q., M.S. Kinch, T.H. Lin, K. Burridge, and R.L. Juliano. 1994. Integrin-mediated cell adhesion activates mitogen-activated protein kinases. *J Biol Chem*. 269:26602-5.

Chen, Q., T.H. Lin, C.J. Der, and R.L. Juliano. 1996. Integrin-mediated activation of MEK and mitogen-activated protein kinase is independent of Ras [corrected]. *J Biol Chem*. 271:18122-7.

Chen, X., Y. Chi, A. Bloecher, R. Aebersold, B.E. Clurman, and J.M. Roberts. 2004. N-acetylation and ubiquitin-independent proteasomal degradation of p21(Cip1). *Mol Cell*. 16:839-47.

Chin, L. 2003. The genetics of malignant melanoma: lessons from mouse and man. *Nat Rev Cancer*. 3:559-70.

Chipuk, J.E., and D.R. Green. 2005. Do inducers of apoptosis trigger caspase-independent cell death? *Nat Rev Mol Cell Biol*. 6:268-75.

Chipuk, J.E., T. Kuwana, L. Bouchier-Hayes, N.M. Droin, D.D. Newmeyer, M. Schuler, and D.R. Green. 2004. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science*. 303:1010-4.

Chipuk, J.E., U. Maurer, D.R. Green, and M. Schuler. 2003. Pharmacologic activation of p53 elicits Bax-dependent apoptosis in the absence of transcription. *Cancer Cell*. 4:371-81.

Clark, E.A., W.G. King, J.S. Brugge, M. Symons, and R.O. Hynes. 1998. Integrin-mediated signals regulated by members of the rho family of GTPases. *J Cell Biol*. 142:573-86.

Clark, W.H., Jr., D.E. Elder, D.t. Guerry, M.N. Epstein, M.H. Greene, and M. Van Horn. 1984. A study of tumor progression: the precursor lesions of superficial spreading and nodular melanoma. *Hum Pathol*. 15:1147-65.

Cobb, M.H., and E.J. Goldsmith. 1995. How MAP kinases are regulated. *J Biol Chem*. 270:14843-6.

Coleman, M.L., C.J. Marshall, and M.F. Olson. 2004. RAS and RHO GTPases in G1-phase cell-cycle regulation. *Nat Rev Mol Cell Biol*. 5:355-66.

Conner, S.R., G. Scott, and A.E. Aplin. 2003. Adhesion-dependent activation of the ERK1/2 cascade is by-passed in melanoma cells. *J Biol Chem*. 278:34548-54.

Cook, A., and J. Milner. 1990. Evidence for allosteric variants of wild-type p53, a tumour suppressor protein. *Br J Cancer*. 61:548-52.

Cukierman, E., R. Pankov, D.R. Stevens, and K.M. Yamada. 2001. Taking cell-matrix adhesions to the third dimension. *Science*. 294:1708-12.

Cukierman, E., R. Pankov, and K.M. Yamada. 2002. Cell interactions with three-dimensional matrices. *Curr Opin Cell Biol*. 14:633-9.

Davies, H.e.a. 2002. Mutations of the BRAF gene in human cancer. *Nature*. 417:949-54.

Davis, G.E. 1992. Affinity of integrins for damaged extracellular matrix: alpha v beta 3 binds to denatured collagen type I through RGD sites. *Biochem Biophys Res Commun*. 182:1025-31.

del Pozo, M.A., L.S. Price, N.B. Alderson, X.D. Ren, and M.A. Schwartz. 2000. Adhesion to the extracellular matrix regulates the coupling of the small GTPase Rac to its effector PAK. *Embo J*. 19:2008-14.

Ding, H.F., Y.L. Lin, G. McGill, P. Juo, H. Zhu, J. Blenis, J. Yuan, and D.E. Fisher. 2000. Essential role for caspase-8 in transcription-independent apoptosis triggered by p53. *J Biol Chem*. 275:38905-11.

Eblen, S.T., J.K. Slack, M.J. Weber, and A.D. Catling. 2002. Rac-PAK signaling stimulates extracellular signal-regulated kinase (ERK) activation by regulating formation of MEK1-ERK complexes. *Mol Cell Biol*. 22:6023-33.

Egan, S.E., and R.A. Weinberg. 1993. The pathway to signal achievement. *Nature*. 365:781-3.

Eliceiri, B.P., R. Klemke, S. Strömblad, and D.A. Cheresh. 1998. Integrin alphavbeta3 requirement for sustained mitogen-activated protein kinase activity during angiogenesis. *J Cell Biol*. 140:1255-63.

Elsdale, T., and J. Bard. 1972. Collagen substrata for studies on cell behavior. *J Cell Biol*. 54:626-37.

Fang, F., G. Orend, N. Watanabe, T. Hunter, and E. Ruoslahti. 1996. Dependence of cyclin E-CDK2 kinase activity on cell anchorage. *Science*. 271:499-502.

Felding-Habermann, B., B.M. Mueller, C.A. Romerdahl, and D.A. Cheresh. 1992. Involvement of integrin alpha V gene expression in human melanoma tumorigenicity. *J Clin Invest*. 89:2018-22.

Foster, B.A., H.A. Coffey, M.J. Morin, and F. Rastinejad. 1999. Pharmacological rescue of mutant p53 conformation and function. *Science*. 286:2507-10.

Fridman, J.S., and S.W. Lowe. 2003. Control of apoptosis by p53. *Oncogene*. 22:9030-40.

Friedl, P., and E.B. Brocker. 2000. The biology of cell locomotion within three-dimensional extracellular matrix. *Cell Mol Life Sci*. 57:41-64.

Friedler, A., L.O. Hansson, D.B. Veprintsev, S.M. Freund, T.M. Rippin, P.V. Nikolova, M.R. Proctor, S. Rudiger, and A.R. Fersht. 2002. A peptide that binds and stabilizes p53 core domain: chaperone strategy for rescue of oncogenic mutants. *Proc Natl Acad Sci U S A*. 99:937-42.

Frisch, S.M., and H. Francis. 1994. Disruption of epithelial cell-matrix interactions induces apoptosis. *J Cell Biol*. 124:619-26.

Frisch, S.M., and R.A. Screaton. 2001. Anoikis mechanisms. *Curr Opin Cell Biol*. 13:555-62.

Frisch, S.M., K. Vuori, D. Kelaita, and S. Sicks. 1996a. A role for Jun-N-terminal kinase in anoikis; suppression by bcl-2 and crmA. *J Cell Biol*. 135:1377-82.

Frisch, S.M., K. Vuori, E. Ruoslahti, and P.Y. Chan-Hui. 1996b. Control of adhesion-dependent cell survival by focal adhesion kinase. *J Cell Biol*. 134:793-9.

Frost, J.A., H. Steen, P. Shapiro, T. Lewis, N. Ahn, P.E. Shaw, and M.H. Cobb. 1997. Cross-cascade activation of ERKs and ternary complex factors by Rho family proteins. *Embo J*. 16:6426-38.

Fuchs, S.Y., V. Adler, T. Buschmann, Z. Yin, X. Wu, S.N. Jones, and Z. Ronai. 1998. JNK targets p53 ubiquitination and degradation in nonstressed cells. *Genes Dev*. 12:2658-63.

Fulda, S., E. Meyer, C. Friesen, S.A. Susin, G. Kroemer, and K.M. Debatin. 2001. Cell type specific involvement of death receptor and mitochondrial pathways in drug-induced apoptosis. *Oncogene*. 20:1063-75.

Fynan, T.M., and M. Reiss. 1993. Resistance to inhibition of cell growth by transforming growth factor-beta and its role in oncogenesis. *Crit Rev Oncog*. 4:493-540.

Gad, A., M. Thullberg, J.H. Dannenberg, H. te Riele, and S. Stromblad. 2004. Retinoblastoma susceptibility gene product (pRb) and p107 functionally separate the requirements for serum and anchorage in the cell cycle G1-phase. *J Biol Chem*. 279:13640-4.

Gannon, J.V., R. Greaves, R. Iggo, and D.P. Lane. 1990. Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for the mutant form. *Embo J*. 9:1595-602.

Garrod, D.R. 1993. Desmosomes and hemidesmosomes. *Curr Opin Cell Biol*. 5:30-40.

Gartel, A.L., and A.L. Tyner. 1999. Transcriptional regulation of the p21((WAF1/CIP1)) gene. *Exp Cell Res*. 246:280-9.
Geiger, B., and A. Bershadsky. 2002. Exploring the neighborhood: adhesion-coupled cell mechanosensors. *Cell*. 110:139-42.
Geiger, B., A. Bershadsky, R. Pankov, and K.M. Yamada. 2001. Transmembrane crosstalk between the extracellular matrix--cytoskeleton crosstalk. *Nat Rev Mol Cell Biol*. 2:793-805.
Giancotti, F.G., and E. Ruoslahti. 1999. Integrin signaling. *Science*. 285:1028-32.
Ginsberg, M.H., X. Du, and E.F. Plow. 1992. Inside-out integrin signalling. *Curr Opin Cell Biol*. 4:766-71.
Gordon, M.K., and B.R. Olsen. 1990. The contribution of collagenous proteins to tissue-specific matrix assemblies. *Curr Opin Cell Biol*. 2:833-8.
Green, D.R. 2000. Apoptotic pathways: paper wraps stone blunts scissors. *Cell*. 102:1-4.
Guan, J.L., J.E. Trevithick, and R.O. Hynes. 1991. Fibronectin/integrin interaction induces tyrosine phosphorylation of a 120-kDa protein. *Cell Regul*. 2:951-64.
Hanahan, D., and R.A. Weinberg. 2000. The hallmarks of cancer. *Cell*. 100:57-70.
Hanks, S.K., M.B. Calalb, M.C. Harper, and S.K. Patel. 1992. Focal adhesion protein-tyrosine kinase phosphorylated in response to cell attachment to fibronectin. *Proc Natl Acad Sci U S A*. 89:8487-91.
Harper, J.W. 2004. Neddylating the guardian; Mdm2 catalyzed conjugation of Nedd8 to p53. *Cell*. 118:2-4.
Hart, M.J., A. Eva, T. Evans, S.A. Aaronson, and R.A. Cerione. 1991. Catalysis of guanine nucleotide exchange on the CDC42Hs protein by the dbl oncogene product. *Nature*. 354:311-4.
Haupt, Y., R. Maya, A. Kazaz, and M. Oren. 1997. Mdm2 promotes the rapid degradation of p53. *Nature*. 387:296-9.
Hegerfeldt, Y., M. Tusch, E.B. Brocker, and P. Friedl. 2002. Collective cell movement in primary melanoma explants: plasticity of cell-cell interaction, beta1-integrin function, and migration strategies. *Cancer Res*. 62:2125-30.
Heino, J. 2000. The collagen receptor integrins have distinct ligand recognition and signaling functions. *Matrix Biol*. 19:319-23.
Hershko, A., and A. Ciechanover. 1998. The ubiquitin system. *Annu Rev Biochem*. 67:425-79.
Hill, C.S., and R. Treisman. 1995. Transcriptional regulation by extracellular signals: mechanisms and specificity. *Cell*. 80:199-211.
Hirai, A., S. Nakamura, Y. Noguchi, T. Yasuda, M. Kitagawa, I. Tatsuno, T. Oeda, K. Tahara, T. Terano, S. Narumiya, L.D. Kohn, and Y. Saito. 1997. Geranylgeranylated rho small GTPase(s) are essential for the degradation of p27Kip1 and facilitate the progression from G1 to S phase in growth-stimulated rat FRTL-5 cells. *J Biol Chem*. 272:13-6.
Hochstrasser, M. 1996. Ubiquitin-dependent protein degradation. *Annu Rev Genet*. 30:405-39.
Hofmann, F., F. Martelli, D.M. Livingston, and Z. Wang. 1996. The retinoblastoma gene product protects E2F-1 from degradation by the ubiquitin-proteasome pathway. *Genes Dev*. 10:2949-59.
Hollstein, M., M. Hergenhahn, Q. Yang, H. Bartsch, Z.Q. Wang, and P. Hainaut. 1999. New approaches to understanding p53 gene tumor mutation spectra. *Mutat Res*. 431:199-209.
Hood, J.D., R. Frausto, W.B. Kiosses, M.A. Schwartz, and D.A. Cheresh. 2003. Differential alphav integrin-mediated Ras-ERK signaling during two pathways of angiogenesis. *J Cell Biol*. 162:933-43.
Howe, A., A.E. Aplin, S.K. Alahari, and R.L. Juliano. 1998. Integrin signaling and cell growth control. *Curr Opin Cell Biol*. 10:220-31.
Howe, A.K., A.E. Aplin, and R.L. Juliano. 2002. Anchorage-dependent ERK signaling--mechanisms and consequences. *Curr Opin Genet Dev*. 12:30-5.

Hsu, M.Y., D.T. Shih, F.E. Meier, P. Van Belle, J.Y. Hsu, D.E. Elder, C.A. Buck, and M. Herlyn. 1998. Adenoviral gene transfer of beta3 integrin subunit induces conversion from radial to vertical growth phase in primary human melanoma. *Am J Pathol*. 153:1435-42.

Huang, S., C.S. Chen, and D.E. Ingber. 1998. Control of cyclin D1, p27(Kip1), and cell cycle progression in human capillary endothelial cells by cell shape and cytoskeletal tension. *Mol Biol Cell*. 9:3179-93.

Humphries, M.J. 2000. Integrin structure. *Biochem Soc Trans*. 28:311-39.

Hungerford, J.E., M.T. Compton, M.L. Matter, B.G. Hoffstrom, and C.A. Otey. 1996. Inhibition of pp125FAK in cultured fibroblasts results in apoptosis. *J Cell Biol*. 135:1383-90.

Hussussian, C.J., J.P. Struewing, A.M. Goldstein, P.A. Higgins, D.S. Ally, M.D. Sheahan, W.H. Clark, Jr., M.A. Tucker, and N.C. Dracopoli. 1994. Germline p16 mutations in familial melanoma. *Nat Genet*. 8:15-21.

Hynes, R.O. 1990. Fibronectin. *Springer Verlag, New York*:554.

Hynes, R.O. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell*. 69:11-25.

Hynes, R.O. 2002. Integrins: bidirectional, allosteric signaling machines. *Cell*. 110:673-87.

Hynes, R.O. 2004. The emergence of integrins: a personal and historical perspective. *Matrix Biol*. 23:333-40.

Ilic, D., E.A. Almeida, D.D. Schlaepfer, P. Dazin, S. Aizawa, and C.H. Damsky. 1998. Extracellular matrix survival signals transduced by focal adhesion kinase suppress p53-mediated apoptosis. *J Cell Biol*. 143:547-60.

Issaeva, N., P. Bozko, M. Enge, M. Protopopova, L.G. Verhoef, M. Masucci, A. Pramanik, and G. Selivanova. 2004. Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. *Nat Med*. 10:1321-8.

Iyer, N.G., S.F. Chin, H. Ozdag, Y. Daigo, D.E. Hu, M. Cariati, K. Brindle, S. Aparicio, and C. Caldas. 2004. p300 regulates p53-dependent apoptosis after DNA damage in colorectal cancer cells by modulation of PUMA/p21 levels. *Proc Natl Acad Sci U S A*. 101:7386-91.

Jacks, T., and R.A. Weinberg. 2002. Taking the study of cancer cell survival to a new dimension. *Cell*. 111:923-5.

Jaffe, A.B., and A. Hall. 2002. Rho GTPases in transformation and metastasis. *Adv Cancer Res*. 84:57-80.

Jan, Y., M. Matter, J.T. Pai, Y.L. Chen, J. Pilch, M. Komatsu, E. Ong, M. Fukuda, and E. Ruoslahti. 2004. A mitochondrial protein, Bit1, mediates apoptosis regulated by integrins and Groucho/TLE corepressors. *Cell*. 116:751-62.

Jeffers, J.R., E. Parganas, Y. Lee, C. Yang, J. Wang, J. Brennan, K.H. MacLean, J. Han, T. Chittenden, J.N. Ihle, P.J. McKinnon, J.L. Cleveland, and G.P. Zambetti. 2003. Puma is an essential mediator of p53-dependent and -independent apoptotic pathways. *Cancer Cell*. 4:321-8.

Jenrette, J.M. 1996. Malignant melanoma: the role of radiation therapy revisited. *Semin Oncol*. 23:759-62.

Jiang, X., and X. Wang. 2004. Cytochrome C-mediated apoptosis. *Annu Rev Biochem*. 73:87-106.

Johnstone, R.W., A.A. Ruefli, and S.W. Lowe. 2002. Apoptosis: a link between cancer genetics and chemotherapy. *Cell*. 108:153-64.

Kadler, K. 1994. Extracellular matrix. 1: fibril-forming collagens. *Protein profile.* 1:519-638.

Khosravi-Far, R., P.A. Solski, G.J. Clark, M.S. Kinch, and C.J. Der. 1995. Activation of Rac1, RhoA, and mitogen-activated protein kinases is required for Ras transformation. *Mol Cell Biol*. 15:6443-53.

Khwaja, A., P. Rodriguez-Viciana, S. Wennstrom, P.H. Warne, and J. Downward. 1997. Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. *Embo J*. 16:2783-93.

King, A.J., H. Sun, B. Diaz, D. Barnard, W. Miao, S. Bagrodia, and M.S. Marshall. 1998. The protein kinase Pak3 positively regulates Raf-1 activity through phosphorylation of serine 338. *Nature*. 396:180-3.

King, R.W., R.J. Deshaies, J.M. Peters, and M.W. Kirschner. 1996. How proteolysis drives the cell cycle. *Science*. 274:1652-9.

Kirsch, D.G., and M.B. Kastan. 1998. Tumor-suppressor p53: implications for tumor development and prognosis. *J Clin Oncol*. 16:3158-68.

Kiyokawa, E., Y. Hashimoto, S. Kobayashi, H. Sugimura, T. Kurata, and M. Matsuda. 1998a. Activation of Rac1 by a Crk SH3-binding protein, DOCK180. *Genes Dev*. 12:3331-6.

Kiyokawa, E., Y. Hashimoto, T. Kurata, H. Sugimura, and M. Matsuda. 1998b. Evidence that DOCK180 up-regulates signals from the CrkII-p130(Cas) complex. *J Biol Chem*. 273:24479-84.

Knupp, C., and J.M. Squire. 2003. Molecular packing in network-forming collagens. *ScientificWorldJournal*. 3:558-77.

Ko, L.J., and C. Prives. 1996. p53: puzzle and paradigm. *Genes Dev*. 10:1054-72.

Koepp, D.M., J.W. Harper, and S.J. Elledge. 1999. How the cyclin became a cyclin: regulated proteolysis in the cell cycle. *Cell*. 97:431-4.

Kozma, R., S. Ahmed, A. Best, and L. Lim. 1995. The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts. *Mol Cell Biol*. 15:1942-52.

LaBaer, J., M.D. Garrett, L.F. Stevenson, J.M. Slingerland, C. Sandhu, H.S. Chou, A. Fattaey, and E. Harlow. 1997. New functional activities for the p21 family of CDK inhibitors. *Genes Dev*. 11:847-62.

Legros, Y., A. Meyer, K. Ory, and T. Soussi. 1994. Mutations in p53 produce a common conformational effect that can be detected with a panel of monoclonal antibodies directed toward the central part of the p53 protein. *Oncogene*. 9:3689-94.

Leu, J.I., P. Dumont, M. Hafey, M.E. Murphy, and D.L. George. 2004. Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. *Nat Cell Biol*. 6:443-50.

Li, W., and H. She. 2000. The SH2 and SH3 adapter Nck: a two-gene family and a linker between tyrosine kinases and multiple signaling networks. *Histol Histopathol*. 15:947-55.

Lin, T.H., A.E. Aplin, Y. Shen, Q. Chen, M. Schaller, L. Romer, I. Aukhil, and R.L. Juliano. 1997a. Integrin-mediated activation of MAP kinase is independent of FAK: evidence for dual integrin signaling pathways in fibroblasts. *J Cell Biol*. 136:1385-95.

Lin, T.H., Q. Chen, A. Howe, and R.L. Juliano. 1997b. Cell anchorage permits efficient signal transduction between ras and its downstream kinases. *J Biol Chem*. 272:8849-52.

Lin, Y., W. Ma, and S. Benchimol. 2000. Pidd, a new death-domain-containing protein, is induced by p53 and promotes apoptosis. *Nat Genet*. 26:122-7.

Lubbe, J., M. Reichel, G. Burg, and P. Kleihues. 1994. Absence of p53 gene mutations in cutaneous melanoma. *J Invest Dermatol*. 102:819-21.

Lukashev, M.E., and Z. Werb. 1998. ECM signalling: orchestrating cell behaviour and misbehaviour. *Trends Cell Biol*. 8:437-41.

Maecker, H.L., C. Koumenis, and A.J. Giaccia. 2000. p53 promotes selection for Fas-mediated apoptotic resistance. *Cancer Res*. 60:4638-44.

Malliri, A., and J.G. Collard. 2003. Role of Rho-family proteins in cell adhesion and cancer. *Curr Opin Cell Biol*. 15:583-9.

Manser, E., T.H. Loo, C.G. Koh, Z.S. Zhao, X.Q. Chen, L. Tan, I. Tan, T. Leung, and L. Lim. 1998. PAK kinases are directly coupled to the PIX family of nucleotide exchange factors. *Mol Cell*. 1:183-92.

Marshall, C.J. 1995. Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell*. 80:179-85.

Matter, M.L., and E. Ruoslahti. 2001. A signaling pathway from the alpha5beta1 and alpha(v)beta3 integrins that elevates bcl-2 transcription. *J Biol Chem*. 276:27757-63.
Meier, F., K. Satyamoorthy, M. Nesbit, M.Y. Hsu, B. Schittek, C. Garbe, and M. Herlyn. 1998. Molecular events in melanoma development and progression. *Front Biosci*. 3:D1005-10.
Meredith, J.E., Jr., B. Fazeli, and M.A. Schwartz. 1993. The extracellular matrix as a cell survival factor. *Mol Biol Cell*. 4:953-61.
Mettouchi, A., S. Klein, W. Guo, M. Lopez-Lago, E. Lemichez, J.K. Westwick, and F.G. Giancotti. 2001. Integrin-specific activation of Rac controls progression through the G(1) phase of the cell cycle. *Mol Cell*. 8:115-27.
Mihara, M., S. Erster, A. Zaika, O. Petrenko, T. Chittenden, P. Pancoska, and U.M. Moll. 2003. p53 has a direct apoptogenic role at the mitochondria. *Mol Cell*. 11:577-90.
Miranti, C.K., and J.S. Brugge. 2002. Sensing the environment: a historical perspective on integrin signal transduction. *Nat Cell Biol*. 4:E83-90.
Miyashita, T., M. Harigai, M. Hanada, and J.C. Reed. 1994. Identification of a p53-dependent negative response element in the bcl-2 gene. *Cancer Res*. 54:3131-5.
Montano, X., M. Shamsher, P. Whitehead, K. Dawson, and J. Newton. 1994. Analysis of p53 in human cutaneous melanoma cell lines. *Oncogene*. 9:1455-9.
Montgomery, A.M., R.A. Reisfeld, and D.A. Cheresh. 1994. Integrin alpha v beta 3 rescues melanoma cells from apoptosis in three-dimensional dermal collagen. *Proc Natl Acad Sci U S A*. 91:8856-60.
Nakano, K., and K.H. Vousden. 2001. PUMA, a novel proapoptotic gene, is induced by p53. *Mol Cell*. 7:683-94.
Nishida, E., and Y. Gotoh. 1993. The MAP kinase cascade is essential for diverse signal transduction pathways. *Trends Biochem Sci*. 18:128-31.
Nobes, C.D., and A. Hall. 1999. Rho GTPases control polarity, protrusion, and adhesion during cell movement. *J Cell Biol*. 144:1235-44.
Obermeier, A., S. Ahmed, E. Manser, S.C. Yen, C. Hall, and L. Lim. 1998. PAK promotes morphological changes by acting upstream of Rac. *Embo J*. 17:4328-39.
Oda, E., R. Ohki, H. Murasawa, J. Nemoto, T. Shibue, T. Yamashita, T. Tokino, T. Taniguchi, and N. Tanaka. 2000a. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science*. 288:1053-8.
Oda, K., H. Arakawa, T. Tanaka, K. Matsuda, C. Tanikawa, T. Mori, H. Nishimori, K. Tamai, T. Tokino, Y. Nakamura, and Y. Taya. 2000b. p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. *Cell*. 102:849-62.
Olson, M.F., H.F. Paterson, and C.J. Marshall. 1998. Signals from Ras and Rho GTPases interact to regulate expression of p21Waf1/Cip1. *Nature*. 394:295-9.
O'Neill, C., P. Jordan, and G. Ireland. 1986. Evidence for two distinct mechanisms of anchorage stimulation in freshly explanted and 3T3 Swiss mouse fibroblasts. *Cell*. 44:489-96.
Oren, M. 1999. Regulation of the p53 tumor suppressor protein. *J Biol Chem*. 274:36031-4.
Owen-Schaub, L.B., W. Zhang, J.C. Cusack, L.S. Angelo, S.M. Santee, T. Fujiwara, J.A. Roth, A.B. Deisseroth, W.W. Zhang, E. Kruzel, and et al. 1995. Wild-type human p53 and a temperature-sensitive mutant induce Fas/APO-1 expression. *Mol Cell Biol*. 15:3032-40.
Pagano, M., S.W. Tam, A.M. Theodoras, P. Beer-Romero, G. Del Sal, V. Chau, P.R. Yew, G.F. Draetta, and M. Rolfe. 1995. Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. *Science*. 269:682-5.
Parsons, J.T. 2003. Focal adhesion kinase: the first ten years. *J Cell Sci*. 116:1409-16.
Petitclerc, E., S. Strömblad, T.L. von Schalscha, F. Mitjans, J. Piulats, A.M. Montgomery, D.A. Cheresh, and P.C. Brooks. 1999. Integrin alpha(v)beta3 promotes M21 melanoma growth in human skin by regulating tumor cell survival. *Cancer Res*. 59:2724-30.

Polte, T.R., and S.K. Hanks. 1997. Complexes of focal adhesion kinase (FAK) and Crk-associated substrate (p130(Cas)) are elevated in cytoskeleton-associated fractions following adhesion and Src transformation. Requirements for Src kinase activity and FAK proline-rich motifs. *J Biol Chem*. 272:5501-9.

Price, L.S., J. Leng, M.A. Schwartz, and G.M. Bokoch. 1998. Activation of Rac and Cdc42 by integrins mediates cell spreading. *Mol Biol Cell*. 9:1863-71.

Raftopoulou, M., and A. Hall. 2004. Cell migration: Rho GTPases lead the way. *Dev Biol*. 265:23-32.

Ravanti, L., J. Heino, C. Lopez-Otin, and V.M. Kahari. 1999. Induction of collagenase-3 (MMP-13) expression in human skin fibroblasts by three-dimensional collagen is mediated by p38 mitogen-activated protein kinase. *J Biol Chem*. 274:2446-55.

Ren, X.D., W.B. Kiosses, and M.A. Schwartz. 1999. Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. *Embo J*. 18:578-85.

Renshaw, M.W., X.D. Ren, and M.A. Schwartz. 1997. Growth factor activation of MAP kinase requires cell adhesion. *Embo J*. 16:5592-9.

Richardson, A., and T. Parsons. 1996. A mechanism for regulation of the adhesion-associated proteintyrosine kinase pp125FAK. *Nature*. 380:538-40.

Ridley, A.J., and A. Hall. 1992. The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell*. 70:389-99.

Roovers, K., G. Davey, X. Zhu, M.E. Bottazzi, and R.K. Assoian. 1999. Alpha5beta1 integrin controls cyclin D1 expression by sustaining mitogen-activated protein kinase activity in growth factor-treated cells. *Mol Biol Cell*. 10:3197-204.

Ruoslahti, E. 1996. RGD and other recognition sequences for integrins. *Annu Rev Cell Dev Biol*. 12:697-715.

Ruoslahti, E., and M.D. Pierschbacher. 1986. Arg-Gly-Asp: a versatile cell recognition signal. *Cell*. 44:517-8.

Ruoslahti, E., and J.C. Reed. 1994. Anchorage dependence, integrins, and apoptosis. *Cell*. 77:477-8.

Rusciano, D. 2000. Differentiation and metastasis in melanoma. *Crit Rev Oncog*. 11:147-63.

Sabapathy, K., M. Klemm, R. Jaenisch, and E.F. Wagner. 1997. Regulation of ES cell differentiation by functional and conformational modulation of p53. *Embo J*. 16:6217-29.

Satyamoorthy, K., T. Bogenrieder, and M. Herlyn. 2001. No longer a molecular black box--new clues to apoptosis and drug resistance in melanoma. *Trends Mol Med*. 7:191-4.

Satyamoorthy, K., N.H. Chehab, M.J. Waterman, M.C. Lien, W.S. El-Deiry, M. Herlyn, and T.D. Halazonetis. 2000. Aberrant regulation and function of wild-type p53 in radioresistant melanoma cells. *Cell Growth Differ*. 11:467-74.

Satyamoorthy, K., G. Li, M.R. Gerrero, M.S. Brose, P. Volpe, B.L. Weber, P. Van Belle, D.E. Elder, and M. Herlyn. 2003. Constitutive mitogen-activated protein kinase activation in melanoma is mediated by both BRAF mutations and autocrine growth factor stimulation. *Cancer Res*. 63:756-9.

Sax, J.K., P. Fei, M.E. Murphy, E. Bernhard, S.J. Korsmeyer, and W.S. El-Deiry. 2002. BID regulation by p53 contributes to chemosensitivity. *Nat Cell Biol*. 4:842-9.

Scatena, M., M. Almeida, M.L. Chaisson, N. Fausto, R.F. Nicosia, and C.M. Giachelli. 1998. NF-kappaB mediates alphavbeta3 integrin-induced endothelial cell survival. *J Cell Biol*. 141:1083-93.

Schaller, M.D., C.A. Borgman, B.S. Cobb, R.R. Vines, A.B. Reynolds, and J.T. Parsons. 1992. pp125FAK a structurally distinctive protein-tyrosine kinase associated with focal adhesions. *Proc Natl Acad Sci U S A*. 89:5192-6.

Schaller, M.D., J.D. Hildebrand, J.D. Shannon, J.W. Fox, R.R. Vines, and J.T. Parsons. 1994. Autophosphorylation of the focal adhesion kinase, pp125FAK, directs SH2-dependent binding of pp60src. *Mol Cell Biol*. 14:1680-8.

Scheffner, M., J.M. Huibregtse, R.D. Vierstra, and P.M. Howley. 1993. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell*. 75:495-505.
Schlaepfer, D.D., S.K. Hanks, T. Hunter, and P. van der Geer. 1994. Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. *Nature*. 372:786-91.
Schlaepfer, D.D., and T. Hunter. 1997. Focal adhesion kinase overexpression enhances ras-dependent integrin signaling to ERK2/mitogen-activated protein kinase through interactions with and activation of c-Src. *J Biol Chem*. 272:13189-95.
Schlaepfer, D.D., and T. Hunter. 1998. Integrin signalling and tyrosine phosphorylation: just the FAKs? *Trends Cell Biol*. 8:151-7.
Schlaepfer, D.D., S.K. Mitra, and D. Ilic. 2004. Control of motile and invasive cell phenotypes by focal adhesion kinase. *Biochim Biophys Acta*. 1692:77-102.
Schmidt, A., and A. Hall. 2002. Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. *Genes Dev*. 16:1587-609.
Schulze, A., K. Lehmann, H.B. Jefferies, M. McMahon, and J. Downward. 2001. Analysis of the transcriptional program induced by Raf in epithelial cells. *Genes Dev*. 15:981-94.
Schwarzbauer, J.E., and J.L. Sechler. 1999. Fibronectin fibrillogenesis: a paradigm for extracellular matrix assembly. *Curr Opin Cell Biol*. 11:622-7.
Seftor, R.E. 1998. Role of the beta3 integrin subunit in human primary melanoma progression: multifunctional activities associated with alpha(v)beta3 integrin expression. *Am J Pathol*. 153:1347-51.
Seftor, R.E., E.A. Seftor, and M.J. Hendrix. 1999. Molecular role(s) for integrins in human melanoma invasion. *Cancer Metastasis Rev*. 18:359-75.
Selivanova, G., V. Iotsova, I. Okan, M. Fritsche, M. Strom, B. Groner, R.C. Grafstrom, and K.G. Wiman. 1997. Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. *Nat Med*. 3:632-8.
Sells, M.A., U.G. Knaus, S. Bagrodia, D.M. Ambrose, G.M. Bokoch, and J. Chernoff. 1997. Human p21-activated kinase (Pak1) regulates actin organization in mammalian cells. *Curr Biol*. 7:202-10.
Semino, C.E., J. Kasahara, Y. Hayashi, and S. Zhang. 2004. Entrapment of migrating hippocampal neural cells in three-dimensional peptide nanofiber scaffold. *Tissue Eng*. 10:643-55.
Semino, C.E., J.R. Merok, G.G. Crane, G. Panagiotakos, and S. Zhang. 2003. Functional differentiation of hepatocyte-like spheroid structures from putative liver progenitor cells in three-dimensional peptide scaffolds. *Differentiation*. 71:262-70.
Sheaff, R.J., J.D. Singer, J. Swanger, M. Smitherman, J.M. Roberts, and B.E. Clurman. 2000. Proteasomal turnover of p21Cip1 does not require p21Cip1 ubiquitination. *Mol Cell*. 5:403-10.
Sherr, C.J., and J.M. Roberts. 1995. Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev*. 9:1149-63.
Sherr, C.J., and J.M. Roberts. 1999. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev*. 13:1501-12.
Slack-Davis, J.K., S.T. Eblen, M. Zecevic, S.A. Boerner, A. Tarcsafalvi, H.B. Diaz, M.S. Marshall, M.J. Weber, J.T. Parsons, and A.D. Catling. 2003. PAK1 phosphorylation of MEK1 regulates fibronectin-stimulated MAPK activation. *J Cell Biol*. 162:281-91.
Soengas, M.S., R.M. Alarcon, H. Yoshida, A.J. Giaccia, R. Hakem, T.W. Mak, and S.W. Lowe. 1999. Apaf-1 and caspase-9 in p53-dependent apoptosis and tumor inhibition. *Science*. 284:156-9.
Soengas, M.S., P. Capodieci, D. Polsky, J. Mora, M. Esteller, X. Opitz-Araya, R. McCombie, J.G. Herman, W.L. Gerald, Y.A. Lazebnik, C. Cordon-Cardo, and S.W. Lowe. 2001. Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. *Nature*. 409:207-11.
Sotomayor, M.G., H. Yu, S. Antonia, E.M. Sotomayor, and D.M. Pardoll. 2002. Advances in gene therapy for malignant melanoma. *Cancer Control*. 9:39-48.

Sporn, M.B. 1996. The war on cancer. *Lancet*. 347:1377-81.
Springer, T.A., and J.H. Wang. 2004. The three-dimensional structure of integrins and their ligands, and conformational regulation of cell adhesion. *Adv Protein Chem*. 68:29-63.
Strömblad, S., J.C. Becker, M. Yebra, P.C. Brooks, and D.A. Cheresh. 1996. Suppression of p53 activity and p21WAF1/CIP1 expression by vascular cell integrin alphaVbeta3 during angiogenesis. *J Clin Invest*. 98:426-33.
Strömblad, S., and D.A. Cheresh. 1996. Integrins, angiogenesis and vascular cell survival. *Chem Biol*. 3:881-5.
Strömblad, S., A. Fotedar, H. Brickner, C. Theesfeld, E. Aguilar de Diaz, M. Friedlander, and D.A. Cheresh. 2002. Loss of p53 compensates for alpha v-integrin function in retinal neovascularization. *J Biol Chem*. 277:13371-4.
Stupack, D.G., X.S. Puente, S. Boutsaboualoy, C.M. Storgard, and D.A. Cheresh. 2001. Apoptosis of adherent cells by recruitment of caspase-8 to unligated integrins. *J Cell Biol*. 155:459-70.
Suzuki, Y., Y. Imai, H. Nakayama, K. Takahashi, K. Takio, and R. Takahashi. 2001. A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. *Mol Cell*. 8:613-21.
Takagi, J., and T.A. Springer. 2002. Integrin activation and structural rearrangement. *Immunol Rev*. 186:141-63.
Takuwa, N., Y. Fukui, and Y. Takuwa. 1999. Cyclin D1 expression mediated by phosphatidylinositol 3-kinase through mTOR-p70(S6K)-independent signaling in growth factor-stimulated NIH 3T3 fibroblasts. *Mol Cell Biol*. 19:1346-58.
Touitou, R., J. Richardson, S. Bose, M. Nakanishi, J. Rivett, and M.J. Allday. 2001. A degradation signal located in the C-terminus of p21WAF1/CIP1 is a binding site for the C8 alpha-subunit of the 20S proteasome. *Embo J*. 20:2367-75.
Uhm, J.H., N.P. Dooley, A.P. Kyritsis, J.S. Rao, and C.L. Gladson. 1999. Vitronectin, a glioma-derived extracellular matrix protein, protects tumor cells from apoptotic death. *Clin Cancer Res*. 5:1587-94.
Van Belle, P.A., R. Elenitsas, K. Satyamoorthy, J.T. Wolfe, D.t. Guerry, L. Schuchter, T.J. Van Belle, S. Albelda, P. Tahin, M. Herlyn, and D.E. Elder. 1999. Progression-related expression of beta3 integrin in melanomas and nevi. *Hum Pathol*. 30:562-7.
van der Rest, M., and R. Garrone. 1991. Collagen family of proteins. *Faseb J*. 5:2814-23.
Vassilev, L.T., B.T. Vu, B. Graves, D. Carvajal, F. Podlaski, Z. Filipovic, N. Kong, U. Kammlott, C. Lukacs, C. Klein, N. Fotouhi, and E.A. Liu. 2004. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science*. 303:844-8.
Vlodavsky, I. 1999. *Curr.Protocals.Cell.Biol*. 1:10.4.1.
Vlodavsky, I., A. Levi, I. Lax, Z. Fuks, and J. Schlessinger. 1982. Induction of cell attachment and morphological differentiation in a pheochromocytoma cell line and embryonal sensory cells by the extracellular matrix. *Dev Biol*. 93:285-300.
Vogelstein, B., D. Lane, and A.J. Levine. 2000. Surfing the p53 network. *Nature*. 408:307-10.
Vojtesek, B., H. Dolezalova, L. Lauerova, M. Svitakova, P. Havlis, J. Kovarik, C.A. Midgley, and D.P. Lane. 1995. Conformational changes in p53 analysed using new antibodies to the core DNA binding domain of the protein. *Oncogene*. 10:389-93.
Voura, E.B., R.A. Ramjeesingh, A.M. Montgomery, and C.H. Siu. 2001. Involvement of integrin alpha(v)beta(3) and cell adhesion molecule L1 in transendothelial migration of melanoma cells. *Mol Biol Cell*. 12:2699-710.
Vousden, K.H., and X. Lu. 2002. Live or let die: the cell's response to p53. *Nat Rev Cancer*. 2:594-604.
Vuori, K., H. Hirai, S. Aizawa, and E. Ruoslahti. 1996. Introduction of p130cas signaling complex formation upon integrin-mediated cell adhesion: a role for Src family kinases. *Mol Cell Biol*. 16:2606-13.

Wang, F., V.M. Weaver, O.W. Petersen, C.A. Larabell, S. Dedhar, P. Briand, R. Lupu, and M.J. Bissell. 1998. Reciprocal interactions between beta1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: a different perspective in epithelial biology. *Proc Natl Acad Sci U S A*. 95:14821-6.

Wang, W., F. Rastinejad, and W.S. El-Deiry. 2003. Restoring p53-dependent tumor suppression. *Cancer Biol Ther*. 2:S55-63.

Wang, X. 2001. The expanding role of mitochondria in apoptosis. *Genes Dev*. 15:2922-33.

Weaver, V.M., A.R. Howlett, B. Langton-Webster, O.W. Petersen, and M.J. Bissell. 1995. The development of a functionally relevant cell culture model of progressive human breast cancer. *Semin Cancer Biol*. 6:175-84.

Weaver, V.M., S. Lelievre, J.N. Lakins, M.A. Chrenek, J.C. Jones, F. Giancotti, Z. Werb, and M.J. Bissell. 2002. beta4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. *Cancer Cell*. 2:205-16.

Wiseman, B.S., and Z. Werb. 2002. Stromal effects on mammary gland development and breast cancer. *Science*. 296:1046-9.

Wozniak, M.A., K. Modzelewska, L. Kwong, and P.J. Keely. 2004. Focal adhesion regulation of cell behavior. *Biochim Biophys Acta*. 1692:103-19.

Wu, X., J.H. Bayle, D. Olson, and A.J. Levine. 1993. The p53-mdm-2 autoregulatory feedback loop. *Genes Dev*. 7:1126-32.

Wyllie, A.H., J.F. Kerr, and A.R. Currie. 1980. Cell death: the significance of apoptosis. *Int Rev Cytol*. 68:251-306.

Xiong, J.P., T. Stehle, B. Diefenbach, R. Zhang, R. Dunker, D.L. Scott, A. Joachimiak, S.L. Goodman, and M.A. Arnaout. 2001. Crystal structure of the extracellular segment of integrin alpha Vbeta3. *Science*. 294:339-45.

Xiong, J.P., T. Stehle, S.L. Goodman, and M.A. Arnaout. 2003. New insights into the structural basis of integrin activation. *Blood*. 102:1155-9.

Xu, L.H., L.V. Owens, G.C. Sturge, X. Yang, E.T. Liu, R.J. Craven, and W.G. Cance. 1996. Attenuation of the expression of the focal adhesion kinase induces apoptosis in tumor cells. *Cell Growth Differ*. 7:413-8.

Yamamoto, M., N. Marui, T. Sakai, N. Morii, S. Kozaki, K. Ikai, S. Imamura, and S. Narumiya. 1993. ADP-ribosylation of the rhoA gene product by botulinum C3 exoenzyme causes Swiss 3T3 cells to accumulate in the G1 phase of the cell cycle. *Oncogene*. 8:1449-55.

Yang, F.C., G. Merlino, and L. Chin. 2001. Genetic dissection of melanoma pathways in the mouse. *Semin Cancer Biol*. 11:261-8.

Zamir, E., and B. Geiger. 2001. Molecular complexity and dynamics of cell-matrix adhesions. *J Cell Sci*. 114:3583-90.

Zamir, E., B.Z. Katz, S. Aota, K.M. Yamada, B. Geiger, and Z. Kam. 1999. Molecular diversity of cell-matrix adhesions. *J Cell Sci*. 112 (Pt 11):1655-69.

Zhang, H., Z. Li, E.K. Viklund, and S. Stromblad. 2002. P21-activated kinase 4 interacts with integrin alpha v beta 5 and regulates alpha v beta 5-mediated cell migration. *J Cell Biol*. 158:1287-97.

Zhang, S., T.C. Holmes, C.M. DiPersio, R.O. Hynes, X. Su, and A. Rich. 1995a. Self-complementary oligopeptide matrices support mammalian cell attachment. *Biomaterials*. 16:1385-93.

Zhang, Z., K. Vuori, J.C. Reed, and E. Ruoslahti. 1995b. The alpha 5 beta 1 integrin supports survival of cells on fibronectin and up-regulates Bcl-2 expression. *Proc Natl Acad Sci U S A*. 92:6161-5.

Zhu, X., M. Ohtsubo, R.M. Bohmer, J.M. Roberts, and R.K. Assoian. 1996. Adhesion-dependent cell cycle progression linked to the expression of cyclin D1, activation of cyclin E-cdk2, and phosphorylation of the retinoblastoma protein. *J Cell Biol*. 133:391-403.