Expert Opinion

1. Introduction
2. Body
3. Conclusion
4. Expert opinion

Therapeutic peptides for cancer therapy. Part I – peptide inhibitors of signal transduction cascades

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Background: Therapeutic peptides have great potential as anticancer agents owing to their ease of rational design and target specificity. However, their utility *in vivo* is limited by low stability and poor tumor penetration. *Objective*: The authors review the development of peptide inhibitors with potential for cancer therapy. Peptides that inhibit signal transduction cascades are discussed. *Methods*: The authors searched Medline for articles concerning the development of therapeutic peptides and their delivery. *Results/conclusion*: Given our current knowledge of protein sequences, structures and interaction interfaces, therapeutic peptides that inhibit interactions of interest are easily designed. These peptides are advantageous because they are highly specific for the interaction of interest, and they are much more easily developed than small molecule inhibitors of the same interactions. The main hurdle to application of peptides for cancer therapy is their poor pharmacokinetic and biodistribution parameters. Therefore, successful development of peptide delivery vectors could potentially make possible the use of this new and very promising class of anticancer agents.

Keywords: c-Myc, drug delivery, Jun-N-terminal kinase, NF-κB, p53, Ras, therapeutic peptide

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1. Introduction

Anticancer peptide therapy is an emerging field that uses bioactive therapeutic peptides (TPs) to kill cancer cells. In the past 15 – 20 years, much effort has been applied to developing peptides capable of eliciting therapeutic responses in cells. Early work was pursued with the goal of using peptides as tools to probe the mechanisms and functional consequences of various protein–protein interactions, but it soon became apparent that peptides capable of mimicking or interfering with important intraprotein contacts could be useful as therapeutic molecules. Peptide therapy has many promising characteristics. First, as opposed to small molecule drugs, peptides are easily designed to target almost any protein of interest using 'rational' methods. As the sequence, structure and interaction partners of many oncogenic proteins are known, peptides can be designed to inhibit these interactions by using a sequence from the interaction domain. Second, peptides are easily produced, and their sequence easily modified using chemical synthesis or molecular biology techniques. However, the utility of peptides for cancer therapy is limited at present by poor pharmacokinetic (PK) parameters and tumor deposition [1,2]. When applied *in vivo*, peptides are rapidly degraded in circulation, and their relatively large size and often charged nature make them impermeable to cancer cell membranes. These limitations can be overcome through the use of non-natural amino acids or macromolecular carriers to enhance peptide stability and through the use of cell penetrating peptides (CPPs) to increase membrane permeability.

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CPPs are a class of peptides capable of delivering large cargo molecules across the plasma membrane. CPPs can be hydrophobic in nature, making them membrane permeable, or, more commonly, highly composed of basic residues. These positively charged peptides have affinity for the outer surface of the plasma membrane, and when bound to cargo they can mediate interaction with the membrane followed by internalization. The mechanism of internalization is unclear, but appears to be dependent on cargo. There is evidence that free peptides or peptides bound to small molecules such as fluorophors can enter cells in an energy-independent non-endocytic process [3], but, when large cargo such as a protein is attached, most data suggest that CPPs enter cells by means of endocytosis [4-6]. The most commonly used strategy for peptide delivery is synthesis of a chimeric peptide containing a CPP fused to a TP, but CPPs can also be fused to macromolecular peptide carriers to enhance their stability and membrane permeability [7,8]. The most commonly used CPPs that are discussed in this review are the penetratin peptide derived from the *Drosophila* antennapedia protein [9], the Tat peptide derived from the HIV-1 Tat protein [10], the MTS (membrane translocating sequence) derived from Kaposi's fibroblast growth factor [11] and the Bac peptide derived from the bovine antibacterial bactenecin peptide [12]. The make-up, entry mechanism and relative efficiency of these peptides have been reviewed previously [13-15].

TPs can be grouped into three classes, those that interfere with proliferative signal transduction cascades, those that arrest the cell cycle by modulating cyclin-dependent kinase activity and those that directly induce apoptosis by modulating proteins that control the apoptotic response. This part of the review focuses on peptide inhibitors of proliferative signalling, and Part II focuses on peptide-based cell cycle inhibitors and apoptosis inducers. The signal transduction peptides function either by inhibiting mitogenic signals (peptides have been described that inhibit several different pathways at various points in each) or by restoring the activity of tumor suppressive proteins such as p53.

2. Body

2.1 Peptide inhibitors of Ras activation

Ras is a small GTPase that is involved in the transduction of signals from cell surface receptors to intracellular signal transduction proteins. There are three Ras genes in humans that encode H-Ras, K-Ras and N-Ras [16]. Ras functions as a binary switch that is activated when bound to GTP and inactivated when bound to GDP [16]. Activation is induced on receipt of a signal from cell surface receptors by guanine nucleotide exchange factors (GEFs) (including SOS1, SOS2 and CDC25), which promote the release of GDP from inactive Ras [17]. Inactivation of Ras occurs when it hydrolyzes GTP to GDP, a reaction that is accelerated by GTPase activating proteins (GAPs) [18]. Active Ras transduces its signal by interacting with Ras effector proteins, including Raf (**Figure 1**) [19]. Ras is one of the most frequently mutated proteins in human cancer, with Ras mutations occurring in 20 – 30% of all cancers [16,20]. Common Ras mutations include alterations at position 12, where a Gly to Val mutation prevents GAPs from activating GTP hydrolysis and renders Ras constitutively active, and at position 61, which in the wild-type protein is a Gln residue that is part of the GTPase active site. Peptide-based strategies have been explored for inhibiting Ras activity by modulating the interaction of Ras with both GEFs and effector proteins.

Inhibition of Ras using peptides from the interaction interfaces of Ras and from several of its binding partners was explored in the lab of Matthew Pincus. This group used molecular dynamics to compare crystal structures of Ras bound to various interaction partners in an attempt to identify regions of both Ras and the interacting proteins that influence binding. Using a *Xenopus laevis* oocyte maturation assay and peptide microinjection, Chung *et al.* [21] identified peptides spanning residues 35 – 47, 96 – 110 and 115 – 126 of Ras as potent inhibitors of Ras-induced oocyte maturation [22]. The 96 – 110 (**Table 1**, peptide 1) and the 115 – 126 peptides were later shown to inhibit oocyte maturation induced by oncogenic Ras, but not by insulin, indicating that they are specific for inhibition of oncogenic Ras. Adler *et al.* demonstrated using pulldown assays that the 96 – 110 peptide (named PNC-2) functions by inhibiting the interaction between Ras and both the transcription factor Jun and its activating kinase Jun-N-terminal kinase (JNK) [23]. The 35 – 47 peptide (named PNC-7) (**Table 1**, peptide 2) inhibited Ras by interfering with its interaction with Raf [24]. Barnard *et al.* also reported that peptides from similar regions of Ras, including amino acids 32 – 37 and 40 – 45, were capable of blocking the Ras–Raf interaction [25]. In an attempt to apply these peptides in a cancer model, Kanovsky *et al.* used normal and Ras-transformed pancreatic cell lines for assays in which the PNC-2 and PNC-7 peptides were fused to the penetratin internalization sequence [26]. When added to the cell culture medium, both penetratin-fused peptides caused phenotypic reversion of the Ras-transformed cells. Furthermore, peptide-treated cells were no longer tumorigenic when injected into nude mice [26]. Importantly, the peptides had no effect on the normal pancreatic cancer cells, indicating that they may specifically inhibit the oncogenic form of Ras. This work was extended by Adler *et al.*, who tested the penetratin-fused PNC-2 and PNC-7 peptides in human HT-1080 fibrosarcoma cells and MIA-PaCa-2 pancreatic cancer cells [27]. Both peptides induced phenotypic reversion in HT-1080 cells as assessed by the inability of the peptide-treated cells to grow in soft agar. In addition, both peptides completely inhibited proliferation of MIA-PaCa-2 cells and induced necrosis in these cells [27].

Using a similar molecular dynamics approach to compare the interaction of the GEF SOS with wild-type and G12V Ras, Chen *et al.* identified four domains of SOS that differed significantly in structure when bound to the normal and

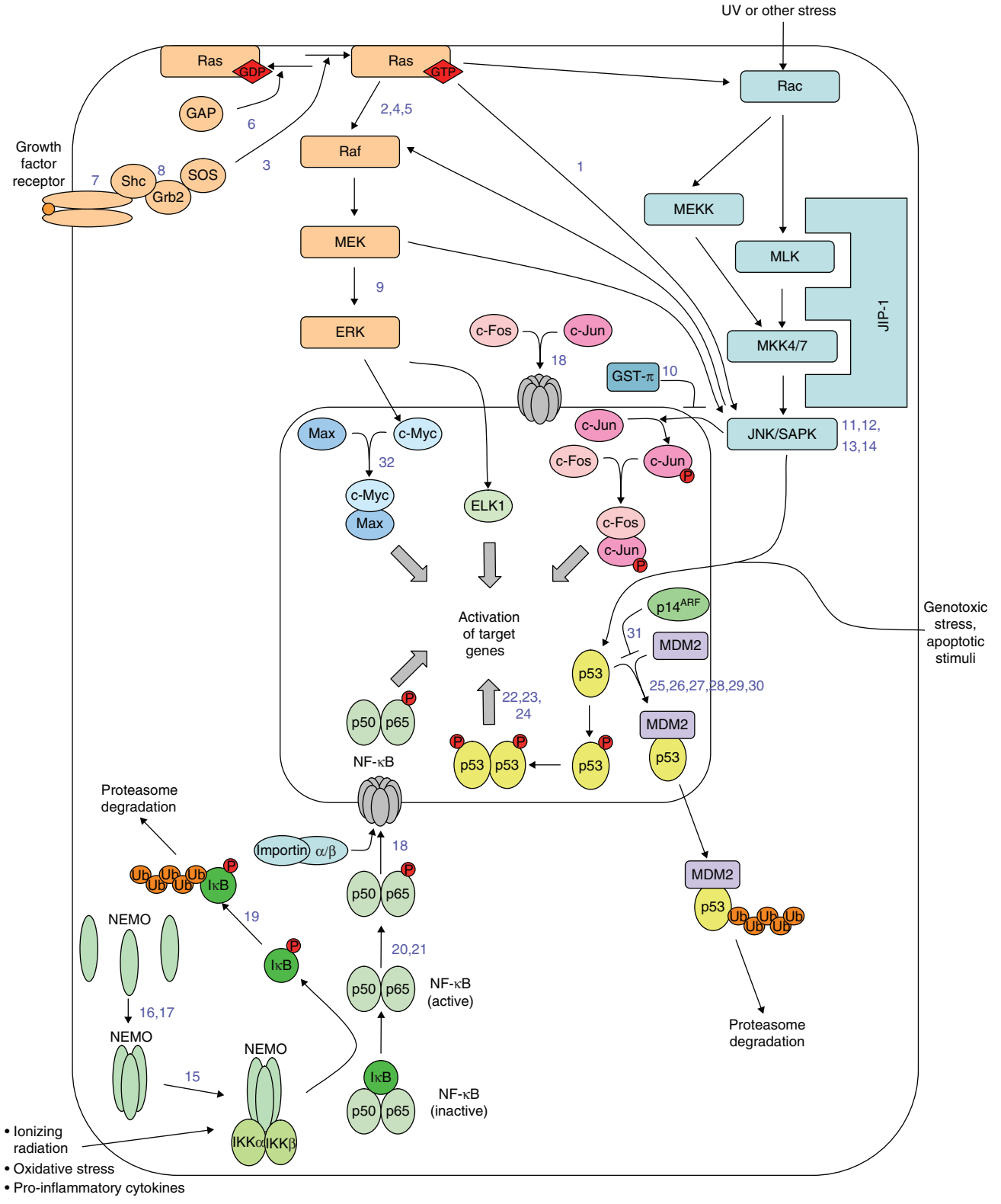

Figure 1. Signal transduction modulating peptides. The major signal transduction cascades that can be inhibited by peptide therapeutics are shown, and the site of action of each peptide is indicated by the numbers, which refer to the peptide number in **Table 1** and in the text.

Table 1. Peptide inhibitors of signal transduction cascades.

Peptide number	Peptide name	Protein of origin	Amino acids	Sequence	Validation	Ref.
1	PNC-2	Ras	96 – 110	YREQIKRVKDSDDVP	*In vitro*	[23]
2	PNC-7	Ras	35 – 47	TIEDSYRKQVVID	*In vitro*	[24]
3	PNC-25	SOS	994 – 1004	LNPMGNSMEKE	*In vitro*	[29]
4	n.s.*	Raf	97 – 110	AVFRLLHEHKGKKA	*In vitro*	[33]
5	n.s.*	Raf	143 – 150	RKTFLKLA	*In vitro*	[34]
6	n.s.*	NF1-GAP	1121 – 1128	RRFFLDIA	*In vitro*	[34]
7	SP1068	EGFR	1063 – 1073	LPVPEpYINQSV‡	*In vitro*	[35]
8	SY317	Shc	312 – 323	FDDPSYVNVQNL	*In vitro*	[37]
9	n.s.*	MEK1	1 – 13	MPKKKPTPIQLNP	*In vitro*	[47]
10	n.s.*	GST-pi	34 – 50	TIDTWMQGLLKPTCLYG	*In vitro*	[49]
11	JNKI1	JIP1/IB1	153 – 172	RPKRPTTLNLFPQVPRSQDT	*In vivo*	[52]
12	JNKI2	JIP2/IB2	134 – 151	HKHRPTTLRLTTLGAQDS	*In vitro*	[52]
13	I-JIP	JIP1/IB1	143 – 163	GPGTGSGDTYRPKRPTTLNLF	*In vitro*	[51]
14	TI-JIP	JIP1/IB1	153 – 163	RPKRPTTLNLF	*In vitro*	[51]
15	NBD	IKKβ	735 – 745	TALDWSWLQTE	*In vivo*	[59]
16	CC2	NEMO	253 – 287	LEDLRQQLQQAEEALVA-KQELIDKLKEEAEQHKIV	*In vitro*	[69]
17	LZ	NEMO	294 – 336	LKAQADIYKADFQAERHAREKL-VEKKEYLQEQLEQLQREFNKL	*In vitro*	[69]
18	SN50	NF-κB p50	360 – 369	VQRKRQKLMP	*In vivo*	[11]
19	pp21	IκBα	21 – 41	KKERLLDDRHDpSGLDpSMKDEE‡	*In vitro*	[81]
20	p65-P1	NF-κB p65	271 – 282	QLRRPSDRELSE	*In vitro*	[82]
21	p65-P6	NF-κB p65	525 – 537	NGLLSGDEDFSS	*In vitro*	[82]
22	C1	p53	369 – 383	LKSKKGQSTSRHKKL	*In vitro*	[83]
23	Peptide 46	p53	361 – 382	GSRAHSSHLKSKKGQSTSRHKK	*In vitro*	[84]
24	CDB3	53BP2	490 – 498	REDEDEIEW	*In vitro*	[89]
25	TIP	p53	12 – 30	PPLSQETFSDLWKLLPENG	*In vitro*	[91]
26	Super-TIP	(selected by phage display)		PPLSMPRFMDYWEGLNENG	*In vitro*	[91]
27	PNC-27	p53	12 – 26	PPLSQETFSDLWKLL	*In vitro*	[93]
28	PNC-21	p53	12 – 20	PPLSQETFS	*In vitro*	[93]
29	PNC-28	p53	17 – 26	ETFSDLWKLL	*In vivo*	[93]
30	αHDM2	p53	16 – 27	QETFSDLWKLLP	*In vivo*	[97]
31	Peptide 3	p14ARF	1 – 20	MVRRFLVTLRIRRACGPPRV	*In vitro*	[98]
32	H1-S6A, F8A	c-Myc	368 – 381	NELKRAFAALRDQI	*In vivo*	[102]

*n.s., name not specified.

‡p denotes a phosphorylated residue.

oncogenic forms of Ras [28]. When peptides from these domains were injected into *Xenopus* oocytes, they all inhibited maturation of the oocytes [29]. However, one domain, from amino acids 994 – 1004 of SOS (**Table 1**, peptide 3), was much more effective at inhibiting oocyte maturation induced by the oncogenic form of Ras [29]. A similar approach was used to identify domains of Ras and GAP that may be involved in Ras signaling [30]. This analysis and subsequent testing using the oocyte maturation assay identified amino acids 66 – 77 and 122 – 138 of Ras as inhibitors of oocyte maturation that were slightly more selective for oncogenic Ras-induced maturation than for wild-type Ras-induced maturation. In addition, five peptides from GAP were shown to inhibit oocyte maturation by both oncogenic Ras and wild-type Ras, and peptides corresponding to amino acids 832 – 845 and 1003 – 1021 of GAP were slightly more selective inhibitors of wild-type Ras-induced oocyte maturation [31].

Chung *et al.* used peptides derived from three regions of the Ras binding domain (RBD) of Raf (previously identified by molecular dynamics analysis as being important for the Ras–Raf interaction [32]) in the *Xenopus* oocyte maturation assay of Ras activation [33]. The authors found that microinjection of all three peptides from the Raf RBD, including residues 62 – 76, 97 – 110 and 111 – 121, blocked oocyte maturation induced by injection of oncogenic Ras or by insulin, and they concluded that the 97 – 110 peptide (**Table 1**, peptide 4) was selective for inhibition of oncogenic Ras-induced oocyte maturation. Barnard *et al.* confirmed that a shorter Raf peptide from the same region, 95 – 101, was capable of blocking the Ras–Raf interaction directly [25].

In a separate approach using sequence alignment of a subset of Ras effector proteins, including Raf family members, NF1-GAP, p120-GAP and others, Clark *et al.* identified a consensus Ras binding sequence in these proteins [34]. Peptides from this sequence, including amino acids 143 – 150 of c-Raf-1 (**Table 1**, peptide 5) and amino acids 1121 – 1128 of NF1-GAP (**Table 1**, peptide 6), blocked Raf-dependent activation of MAP kinases *in vitro* [34].

In an attempt to inhibit the Ras pathway upstream of Ras, Rojas *et al.* examined a phosphopeptide from a region of the epidermal growth factor receptor (EGFR) [35]. EGFR can stimulate Ras activation by means of a network of proteins including the adapter proteins Grb2 and Shc and the GEF SOS. Tyrosine 1068 of EGFR is a known site of autophosphorylation, and it is bound by the SH2 domain of Grb2 [36]. The authors used a peptide containing EGFR amino acids 1063 – 1073 including a phosphorylated Y1068 (**Table 1**, peptide 7) fused to the membrane translocating sequence (MTS) from Kaposi's fibroblast growth factor [11] to inhibit Ras activation. They demonstrated, using coimmunoprecipitation experiments, that this peptide was able to bind to Grb2 and inhibit the interaction of EGFR and Grb2 in EGF-stimulated NIH 3T3 cells overexpressing EGFR [35]. Furthermore, the MTS-EGFR phosphopeptide was able partially to inhibit SOS-mediated nucleotide exchange in Ras and subsequently to inhibit MAP kinase activation. Although this peptide was an effective inhibitor of Ras activation, it required a phosphorylated tyrosine residue, making its synthesis more difficult and potential utility as a therapeutic limited. In a later study, Rojas *et al.* used a non-phosphorylated peptide from Shc to inhibit the Grb2–Shc interaction (**Table 1**, peptide 8) [37]. They demonstrated that amino acids 312 – 323 of Shc fused to MTS were capable of blocking the interaction of Grb2 and Shc, regardless of whether Y317 was phosphorylated.

A final strategy for inhibiting Ras signaling involves using peptide inhibitors of the enzyme farnesyltransferase, which farnesylates the C terminus of Ras and anchors it to the membrane. Peptides that contain the amino acid motif CA_1A_2X (where A is an aliphatic amino acid and X is M or S) have been applied as competitive inhibitors of Ras farnesylation [38-40]. Work with CA_1A_2X peptides has been reviewed previously [41] and, because of the poor performance of farnesyltransferase inhibitors in clinical trials [42,43], this strategy will not be discussed further here.

2.2 Peptide inhibitors of MAP kinases

Extracellular signals initiated by receptor binding of ligands are transmitted to the nucleus through a variety of signal transduction cascades. In general, these cascades include activation of a small G-protein by the receptor and accessory proteins, which in turn activates a protein kinase and triggers a phosphorylation cascade, eventually resulting in activation of a transcription factor. Two of the most studied of these cascades include the ERK (extracellular signal-regulated kinase) pathway [44] and the JNK/SAPK (Jun N-terminal kinase/stress-activated protein kinase) pathway (**Figure 1**) [45]. The ERK pathway is activated by mitogenic signals such as EGF binding EGFR, which leads to Ras activation as described above. Ras then activates the kinase Raf, which phosphorylates MEK, which in turn phosphorylates ERK. The phosphorylated ERK can then enter the nucleus, where it phosphorylates and activates transcription factors such as c-Myc and Elk-1. These transcription factors activate genes responsible for cell proliferation. The JNK/SAPK pathway is activated by heat shock, UV radiation, or inflammatory cytokines. The small G-proteins in this pathway are Rac and cdc42, which initiate a phosphorylation cascade that proceeds through MEKKs or MLKs to MKK4/7 and JNK/SAPK. JNK/SAPK binds the transcription factor c-Jun and activates it by phosphorylation. Active c-Jun functions as a heterodimer with c-Fos. The c-Jun/c-Fos heterodimer (AP1) then activates genes responsible for cell proliferation, response to UV damage, and a variety of other processes. Other targets of JNKs are activating transcription factor 2 (ATF2) and Elk1-1. However, these pathways are not independent of one another. There are points of crosstalk between them. For example, Ras forms a complex with Raf, MEK and JNK [46]; MEK can phosphorylate JNK; and JNK can in turn phosphorylate Raf [46].

Kelemen *et al.* studied peptides from MEK derived from the region known to interact with ERK with the goal of producing an ERK inhibitor [47]. The authors report that a peptide containing the N-terminal 13 amino acids of MEK (**Table 1**, peptide 9) was capable of binding ERK *in vitro*. When the penetratin or Tat CPPs were attached to the peptide, they were able to mediate entry of the peptides into NIH 3T3 cells and PC12 cells. Furthermore, the CPP-fused peptides were able to inhibit ERK activation in the cells, and they subsequently inhibited activation of the transcription factor Elk-1 as determined by an Elk-1 luciferase reporter system.

The Pincus lab also studied ways in which peptides could be used to inhibit the JNK pathway. First, they identified that the enzyme GST-pi blocked the phosphorylation of Jun by JNK [48]. Using a molecular dynamics approach similar to that described above to compare free GST-pi and inhibitor bound GST-pi, Adler and Pincus identified four putative domains that may be involved in the GST-pi–JNK interaction [49]. After analyzing effects of all four peptides on GST binding to Jun–JNK and on Jun phosphorylation, the peptide from amino acids 34 – 50 (**Table 1**, peptide 10) was found to be most effective for inhibiting Jun phosphorylation. Furthermore, when fused to penetratin, the 34 – 50 peptide inhibited the phosphorylation of Jun in the U 291 astrocytoma cell line. The authors conclude that this peptide functions by blocking the phosphorylation of JNK or by promoting the dephosphorylation of active JNK, thereby inhibiting Jun phosphorylation as a downstream event. In a follow-up study, Chie *et al.* determined that the 34 – 50 peptide inhibited oocyte maturation induced by oncogenic Ras, but not by wild-type Ras, indicating that this peptide could be a useful therapeutic in Ras-mutated cancers [50].

Organization of the different MAPK pathways is aided by scaffolding proteins, non-enzymatic proteins that serve as anchoring points for the pathway kinases and substrates. One such scaffolding protein for JNKs is JNK-interacting protein-1 (JIP1). Overexpression of the JNK binding domain (JBD) from JIP-1 has been shown to inhibit the JNK pathway in several model systems (see [51] for summary). Using an alignment of the JBDs of IB-1 and IB-2 (isoforms of JIP-1 and JIP-2), Bonny *et al.* identified peptides of 20 and 18 amino acids from IB-1 (called JNKI-1) and IB-2 (called JNKI-2), respectively (**Table 1**, peptides 11 and 12), that were highly conserved between the two proteins [52]. When the two peptides were fused with Tat, the fusion peptides were able to gain entry into pancreatic β-cells grown in culture. In addition, these peptides were able to inhibit JNK phosphorylation of c-Jun *in vitro* and reduce c-Jun expression in cells. Finally, retro-inverso (RI) forms of the peptides, which were generated by synthesizing the peptide in reverse order (C to N terminus) out of D-amino acids, were capable of inhibiting IL-1β-induced apoptosis in pancreatic β-cells, highlighting their potential for therapy of diabetes. In a subsequent *in vivo* study, Borsello *et al.* demonstrated that the Tat-JIP derived peptide, when injected intraventricularly, was neuroprotective in rat models of middle cerebral artery occlusion [53], highlighting their potential for therapy of stroke. In addition, cell-permeable JNK inhibitory peptides have shown efficacy in a variety of other disease models *in vitro* and *in vivo*, including neurotrama, hearing loss, Alzheimer's disease and asthma (reviewed in [54-56]). Barr *et al.* chose to define the minimal region of the JBD necessary for JNK inhibition. The authors determined that a peptide from amino acids 143 – 163 of JIP1 (**Table 1**, peptide 13), which overlaps with the IB-1 peptide used by Bonny, was capable of inhibiting phosphorylation of c-Jun, Elk-1 and ATF2 *in vitro*, and they further determined that an even shorter version of the peptide (**Table 1**, peptide 14), spanning amino acids 153 – 163, could bind JNK directly (as assessed by surface plasmon resonance) and inhibit JNK activity [51]. Recently, Gao *et al.* evaluated the D-amino acid version of the JIP-1-derived peptide (D-JNKI-1) in a mouse model of melanoma in which cells were injected into the hind paw, and evaluated the effects of the peptide on both tumor growth and tumor-induced pain [57]. When injected intraperitoneally (i.p.) twice a day from day 5 to day 9 after tumor implantation, D-JNKI-1 prevented tumor growth entirely during the days of administration, whereas tumors in control-treated animals doubled over the same time period. In addition to reducing tumor growth, D-JNKI-1 administration also attenuated mechanical allodynia (painful response to a stimulus that is normally not painful) and heat-induced hyperalgesia (extreme reaction to a stimulus that is normally moderately painful).

2.3 Peptide inhibitors of NF-κB activation

NF-κB is a transcriptional regulator that controls expression of numerous genes involved in many cellular processes, including inflammation, proliferation and apoptosis. Owing to the wide variety of genes activated by NF-κB, its dysregulation is associated with a myriad of diseases, including cancer, autoimmune diseases, inflammatory diseases (asthma, arthritis and inflammatory bowel disease) and neurodegenerative diseases (reviewed in [58]). NF-κB family members include p50, p52, p65 (RelA), c-Rel and RelB. NF-κB activation is controlled by regulating its subcellular localization. In the inactive state, NF-κB is bound by IκB (inhibitor of κB) proteins, which mask the nuclear localization sequence (NLS) of NF-κB and sequester it to the cytoplasm. On activation by signals originating from extracellular stimuli, IκB kinase (IKK) is activated, and IKK phosphorylates IκB and marks it for poly-ubiquitination and degradation by the proteasome. Once IκB is degraded, the NF-κB NLS is exposed, and NF-κB is translocated into the nucleus where it regulates gene transcription (**Figure 1**). IKK is composed of three subunits, the IKKα and IKKβ kinase subunits and a master regulatory protein called NEMO (NF-κB essential modulator) [58]. Peptide strategies for inhibition of NF-κB activation include prevention of IKK activation by blocking NEMO–IKK binding, prevention of NEMO trimerization,

directly blocking the phosphorylation of IκB, and prevention of nuclear localization of NF-κB.

The first strategy for inhibiting NF-κB activation involves blocking the interaction between the IKK subunits and their regulator, NEMO, which in turn prevents phosphorylation and degradation of the NF-κB inhibitor IκB. In a seminal study, May *et al*. identified a six-amino acid region from the C terminus of IKKα and IKKβ responsible for NEMO binding, and termed a peptide surrounding this region the NEMO binding domain (NBD) (**Table 1**, peptide 15) [59]. When fused to the penetratin peptide, the NBD was capable of blocking the interaction between NEMO and IKK and preventing NF-κB activation in HeLa cells *in vitro*. The cell-permeable NBD peptide was also effective for reduction of inflammation *in vivo* in a PMA (phorbol 12-myristate 13-acetate)-induced ear edema model and a zymosan-induced peritonitis model. The cell-permeable NBD peptide has since shown efficacy in mouse models of multiple sclerosis [60], inflammation (λ-carrageenan-induced paw edema) [61], arthritis [62] and Parkinson's disease [63]. When fused to an alternative CPP called PTD-5, the NBD peptide was capable of entering pancreatic islet cells both *in vitro* and *in vivo*, and it blocked IL-1β-mediated islet cell dysfunction both in culture and *in vivo* [64], demonstrating efficacy for the NBD peptide for therapy of diabetes. The NBD peptide has also been shown to be useful in several cancer models. In the TRAIL-resistant pancreatic cancer cell line L3.6, the penetratin-fused NBD peptide restored the ability of TRAIL to induce apoptosis and inhibit cell proliferation [65]. When studying NF-κB activation in human breast tumor specimens, Biswas *et al*. determined that NF-κB was activated predominantly in estrogen receptor-negative, ErbB2-positive tumors [66]. Furthermore, the authors showed that the penetratin-fused NBD peptide blocked heregulin-induced NF-κB activation in SKBr3 cells, caused cell cycle arrest in G_0/G_1 phase, and induced apoptotic cell death [66]. The NBD peptide also prevented NF-κB activation as induced by the chemotherapeutic doxorubicin (Dox) and enhanced Dox toxicity in BT-474 breast cancer cells [67]. The NBD peptide directly inhibited the proliferation of the human melanoma cell line A375, in which NF-κB is constitutively active [68].

Another strategy for NF-κB inhibition also involves inhibition of NEMO function, but this peptide does so by inhibiting trimerization of NEMO. Oligomerization of NEMO is essential for its activation of NF-κB. Agou *et al*. demonstrated that NEMO trimerization occurs by means of its C-terminal coiled coil 2–leucine zipper (CC2–LZ) domains [69], and they described two peptides derived from these domains that were capable of preventing NF-κB activation. When fused to the penetratin peptide, a peptide encompassing the CC2 domain (amino acids 253 – 287) (**Table 1**, peptide 16) was capable of reducing LPS-induced NF-κB activation twofold with an IC_{50} of 22 μM. A peptide composed of penetratin fused to a LZ peptide (amino acids 294 – 336) (**Table 1**, peptide 17) was even more potent, causing a 50-fold reduction in LPS-induced NF-κB activity with an IC_{50} of only 3 μM. The LZ peptide interacted with NEMO in cultured cells, and the LZ peptide fused to penetratin was a potent inhibitor of proliferation of the Y79 retinoblastoma cell line [70]. In addition, the LZ peptide fused to polyarginine induced caspase activation and apoptosis in several AML cell lines and in primary cells cultured from AML and high-risk MDS (myelodysplastic syndrome) patients [71]. Excitingly, $CD34^+$ cells from healthy controls were not killed by the peptide, indicating that this therapy might be specific for the precancerous and cancerous cell types that are dependent on NF-κB activity.

Another strategy for inhibiting NF-κB activity involves blocking its nuclear localization after an activating signal is received. On stimulation with a pro-inflammatory signal, IκB is phosphorylated and degraded, and a nuclear localization sequence on the p50 subunit of NF-κB is exposed. Lin *et al*. described a peptide in which the p50 NLS was fused to a hydrophobic cell-penetrating peptide (dubbed SN50, **Table 1**, peptide 18), which is capable of inhibiting the nuclear translocation of NF-κB [11]. Furthermore, this peptide not only inhibits the nuclear localization of NF-κB, but it also blocks nuclear import of other transcription factors, including AP-1, NFAT and STAT1, by binding and sequestering the Rch1/importin-β NLS receptor complex [72]. The SN50 peptide showed preclinical promise in a rat model of acute pancreatitis [73], a mouse model of corneal alkali burns [74] and a mouse model of endometriosis [75]. The SN50 peptide was also effective in several cancer models. The peptide induced apoptosis in Y79 and WERI-Rb1 retinoblastoma cell lines, and it enhanced the potency of Dox in Y79 cells [76]. SN50 also directly inhibited the proliferation of human thyroid cancer cells and enhanced their sensitivity to ionizing radiation [77]. A related peptide, SN52 (which uses the p52 NLS rather than the p50 NLS), sensitized prostate cancer cells to low doses of ionizing radiation [78]. Finally, a cyclized version of the SN50 peptide was shown to be a more potent inhibitor of NF-κB nuclear import than the linear version. This cyclized peptide was taken up by leukocytes, lymphocytes and splenocytes following IP injection in mice, and prevented LPS-induced lethal shock in these animals [79].

In an alternative but related strategy for blocking NF-κB nuclear localization, Fujihara *et al*. fused either the NLS from the SV40 large T antigen or the NLS from c-Myc to both ends of the hydrophobic cell-penetrating peptide. Both peptides were capable of blocking NF-κB nuclear localization in LPS-treated 70Z/3 murine pre-B cell line and in human PBL, and the peptide containing the SV40 NLS protected mice from septic shock and reduced inflammation and lesions in a mouse model of inflammatory bowel disease [80].

Other strategies for blocking NF-κB activation include preventing degradation of IκB and blocking phosphorylation of p65. Yaron *et al*. describe the use of phosphopeptides derived from IκBα (**Table 1**, peptide 19), which were capable of inhibiting the ubiquitination and degradation of IκBα and subsequently blocking TNF-α-induced NF-κB activation

in HeLa cells [81]. Takada *et al.* used synthetic peptides derived from the phosphorylation sites in the DNA binding/dimerization domain or the transactivation domain of p65 for NF-κB inhibition (**Table 1**, peptides 20 and 21). When fused to the penetratin CPP, peptides from both domains effectively blocked TNF-α-induced NF-κB activation in KBM-5 leukemia cells and enhanced TNF-α-induced apoptosis. Furthermore, the peptide derived from the DNA binding/dimerization domain enhanced the potency of both Dox and cisplatin in KBM-5 cells [82].

2.4 Peptides that affect p53 function

p53 is one of the most commonly mutated or deleted genes in human cancers. The p53 protein is a transcription factor that plays a role in the regulation of cell cycle and apoptotic responses to insults such as DNA damage. Therefore, mutations that abolish p53 function confer cells with resistance to apoptotic stimuli. p53 protein levels are controlled by regulation of the degradation rate of the protein. In unstimulated cells, p53 is bound by the ubiquitin E3 ligase MDM-2 and tagged for degradation at the proteasome, resulting in very low steady-state levels. However, on stimulation, such as by DNA damage or oxidative stress, p53 is phosphorylated (by members of the MAPK family discussed above or by protein kinases, such as Chk1, Chk2, CAK, and others), MDM2 binding is abolished, and degradation is halted. p53 can then bind DNA and regulate transcription of its target genes (**Figure 1**), which include the cyclin-dependent kinase inhibitor (CDKI) p21. Owing to the high prevalence of p53 mutation in human cancers and the deleterious results of loss of p53 function, much effort is being applied to developing methods of reactivation p53 function in cancerous tissue. Several peptide strategies have been used with this goal, including peptides that restore the ability of mutant p53 to bind DNA and activate transcription and peptides that block MDM2 binding to p53.

Armed with the knowledge that an antibody specific to a C-terminal epitope on p53 could activate p53 DNA binding, Hupp *et al.* identified a peptide from the C-terminal domain of p53 (amino acids 369 – 383) (**Table 1**, peptide 22) that could induce latent p53 to bind to DNA [83]. Based on this work, a very similar peptide (**Table 1**, peptide 23) (amino acids 361 – 382, dubbed Peptide 46) was fused to the penetratin CPP by Selivanova *et al.*, and they demonstrated that this fusion peptide could activate p53-mediated transcription in cell lines expressing both wild-type p53 and the His-273/Ser-309 mutant p53 [84]. Furthermore, the penetratin-fused Peptide 46 was capable of inducing apoptosis in Ew36 Burkitt lymphoma cells (wild-type p53), SW480 colon carcinoma cells (His-273/Ser-309 mutant p53) and BL41 Burkitt lymphoma cells (Gln-248 mutant p53), but was not toxic to the p53 null cell lines HL60 and Saos-2. The authors determined that this peptide functioned by binding p53 and inducing its binding of DNA [85]. Importantly, Peptide 46 was able to restore the ability of several mutant forms of p53 (His-273 and Trp-248, DNA contact mutants; His-175, Ala-143 and Ser-249, structural mutants) to bind DNA [85]. The penetratin-fused Peptide 46 was later shown to induce apoptosis in MCF-7 (overexpressed wild-type p53), MDA-MB-468 (His-273 mutant p53) and MDA-MB-231 (Lys-280 mutant p53) breast cancer cells, but not in the normal breast epithelial cell line MCF-10-2A [86,87], and in pre-malignant and malignant colon cell lines RG/C2 and BR/C1 (which contain mutant p53), but not the normal colon cell line CCD33Co or the pre-malignant, wild-type p53 containing AA/C1 colon cell line [88]. Use of these C-terminal p53 peptides is a promising strategy for reactivation of mutant p53.

With a similar goal of identifying peptides that can bind and stabilize mutant p53 and/or restore its ability to bind DNA, Friedler *et al.* have described a 9-amino acid peptide, which they called CDB3, derived from the p53 binding protein 53BP2 (amino acids 490 – 498) (**Table 1**, peptide 24) [89]. This peptide was capable of tightly binding the p53 core domain, and also bound the core domain of the Ser-245 mutant and the Ser-249 mutant. CDB3 binding increased the stability of the p53 core domain, and it restored the ability of the highly destabilized Thr-195 mutant to bind DNA. CDB3 was capable of binding to p53 in cultured cells and stabilizing the His-175 and His-273 mutant proteins [90]. Furthermore, treatment of cells with CDB3 led to an upregulation of the p53-controlled genes *p21* and *mdm2*, induced apoptosis in cells carrying p53 mutations, and sensitized cells carrying wild-type p53 to γ-irradiation.

Another strategy for restoration of p53 function involves increasing the levels of p53 by preventing its proteolysis. Several groups have utilized different versions of a peptide derived from the N terminus of p53 to block the interaction of p53 with MDM2, thereby preventing its degradation. In a seminal paper, Böttger *et al.* reported that a peptide containing amino acids 12 – 30 of p53 (**Table 1**, peptide 25), as well as a derivative of this peptide selected by phage display (**Table 1**, peptide 26), were capable of inhibiting the interaction of p53 and MDM2 when fused to thioredoxin as an insertion into the active site loop [91]. When expressed in cells, the mini-protein containing the optimized phage-selected peptide caused an increase in p53 levels and an arrest of the cell cycle. Wasylyk *et al.* [92] expressed this modified p53 peptide in SA1 cells, an osteosarcoma cell line that expresses wild-type p53 and overexpresses MDM2. They found that the peptide led to increased p53 levels and consequently inhibited cell proliferation as assessed by colony formation, cell cycle arrest and apoptosis assays. Using X-ray crystallographic data for the p53–MDM2 interaction and the work of Böttger and Wasylyk, Kanovsky *et al.*, in the lab of Matthew Pincus, described three peptides from the MDM2-binding domain of p53, and they demonstrated that these peptides were cytotoxic to human cancer cells *in vitro* [93]. Based on an X-ray structure that showed that amino acids 12 – 26 of p53 interact with MDM2, the authors generated

three peptides, spanning residues 12 – 26, 12 – 20 and 17 – 26 (**Table 1**, peptides 27 – 29), and fused each to the penetratin CPP. Using the same k-*ras* transformed acinar pancreatic carcinoma cells used for analysis of their Ras inhibitory peptides, the authors demonstrated that all three peptides, and particularly the peptide spanning amino acids 17 – 26, which they dubbed PNC-28, were capable of inducing nearly 100% cell death in the transformed cell line while causing no toxicity to the untransformed parental cell line. In addition, PNC-28 killed the human cancer cell lines HeLa and SW1417, the latter of which raised an interesting question. SW1417 contains a homozygous deletion of the *TP53* gene. Therefore, the ability of PNC-28 to kill these cells indicates that this peptide acts in a p53-independent manner, and the authors speculate that the peptide may interfere with other functions of MDM2 that are unrelated to p53. This work was extended by Do *et al*., who demonstrated that all three peptides were effective inhibitors of breast cancer cell proliferation, with the peptide spanning amino acids 12 – 26 (PNC-27) being the most potent [94]. Furthermore, the peptide also inhibited cell proliferation in the MDA-MB-468, MCF-7 and MDA-MB-157 cell lines, which contain mutant, overexpressed wild type, and deleted p53, respectively, but had no effect on the non-malignant breast epithelial cell line MCF-10-2A. Cell death was confirmed to occur by means of a necrotic mechanism involving cell lysis, and the specificity of the peptide for the cancer cells over the non-malignant cell line was due to enhanced uptake of the peptide by the cancer cells. This necrotic mechanism of cell death was recently confirmed in pancreatic cancer cells, in which the penetratin-fused PNC-28 peptide rapidly induced necrosis through a membrane lytic mechanism, but the peptide containing the p53-derived amino acids without penetratin (the peptide was expressed from a transfected plasmid) caused apoptosis [95]. This highlights an interesting contrast between the peptide described by Böttger and Wasylyk and PNC-28. Although both peptides are derived from the same region of p53, the Böttger and Wasylyk peptide functions by means of a p53-dependent apoptotic mechanism, and PNC-28 functions by means of cell lysis and necrosis. It is the placement of the penetratin peptide at the C terminus and resulting helical structure that makes PNC-28 lytic, and therefore it exerts a toxic effect before it even has a chance to enter the cells and interact with MDM2. The Pincus lab evaluated the PNC-28 peptide further *in vivo* in a mouse model of pancreatic cancer [96]. When PNC-28 was delivered continuously over 14 days by either a subcutaneously (s.c.) or i.p. placed osmotic pump, the peptide was able to prevent the formation of ascites and liver metastases following i.p. injection of k-Ras-transformed pancreatic cancer cells. Also, s.c. delivery of PNC-28 over 14 days slowed the proliferation of the same pancreatic cancer cells when implanted as s.c. xenografts. A separate group also tested a very similar p53 peptide spanning amino acids 16 – 27 (**Table 1**, peptide 30) fused N-terminally with the Tat CPP [97]. This peptide induced cell death in MM-23 uveal melanoma, WERI retinoblastoma, U2OS osteosarcoma and C33A cervical carcinoma cells and, in contrast to the necrotic mechanism demonstrated for PNC-28, cell death was determined to be by means of a p53 and caspase-dependent apoptotic mechanism. Furthermore, after direct intraocular injection, this peptide was able to induce apoptosis in WERI retinoblastoma tumors implanted intraocularly in immunosuppressed white rabbits. These data highlight the importance of choice and placement of the CPP, as the use of Tat at the N terminus (as in Harbour) or fusion to thioredoxin (as in Böttger) or no CPP (as in Wasylyk) lead to a p53-dependent, apoptosis-inducing mechanism, and the use of penetratin at the C terminus results in a peptide with a unique structure that is capable of inducing membrane lysis.

An alternative strategy for inhibition of MDM2-mediated p53 degradation used peptides derived from $p14^{ARF}$. $p14^{ARF}$ is a tumor suppressor protein that inhibits MDM2-mediated ubiquitination of p53. Midgley *et al*. screened 20 amino acid peptides covering the entire sequence of the $p14^{ARF}$ protein, and they determined that amino acids 1 – 20 (termed Peptide 3) (**Table 1**, peptide 31) were capable of binding MDM2 [98]. Peptide 3 blocked the MDM2-mediated ubiquitination of p53 *in vitro*, presumably by serving as a competitive inhibitor of MDM2/p53 binding. When expressed in MCF-7 breast cancer cells and U2OS osteosarcoma cells as a fusion with GFP, Peptide 3 caused an increase in cellular p53 levels. Using an interesting approach to increase intracellular peptide stability, Karlsson *et al*. inserted a related ARF peptide (amino acids 1 – 37 from $p19^{ARF}$, the mouse homolog of human $p14^{ARF}$) as well as the p53 12 – 30 peptide described by Böttger into a scaffold protein derived from chymotrypsin inhibitor 2 [99]. Expression of both scaffold-fused peptides in cells caused an increase in p53 activity, and the authors showed that the scaffold-fused ARF peptide was capable of interacting with MDM2. Finally, both peptide constructs caused cell cycle arrest.

2.5 Peptide inhibitors of c-Myc activation

c-Myc is a transcription factor that regulates the expression of a huge number of genes and is often overexpressed in cancers [100]. c-Myc is a basic helix–loop–helix leucine zipper (bHLH/LZ) containing transcription factor that binds DNA as a heterodimer with Max [101]. c-Myc is activated by mitogenic signals through the Ras/MAPK pathway (**Figure 1**). Genes controlled by c-Myc/Max are widely varied, but they are responsible for such processes as cell cycle control, cell proliferation, cell growth and apoptosis. Consequently, overexpression of c-Myc can lead to unregulated cell proliferation. In cancers, c-Myc dysregulation is often combined with other oncogenic changes, such as Ras activation, which cause cells to grow unchecked. Strategies for inhibition of c-Myc function have focused on blocking its interaction with Max.

In one of the first reports of a peptide inhibitor of a signaling protein, Draeger and Mullen analyzed peptides from

the Helix 1 (H1) and leucine zipper (LZ) regions of both c-Myc and Max to determine their structure and ability to interfere with the c-Myc/Max interaction. None of the wild type peptides studied was active as an inhibitor; but the authors substituted alanines at non-conserved residues in order to confer greater helical content, and two mutated peptides from H1 of c-Myc (H1-F8A and H1-S6A, F8A) were capable of blocking DNA binding by c-Myc *in vitro* [102]. The authors determined that these peptides functioned by binding directly to c-Myc, and the H1-S6A, F8A double mutant peptide (**Table 1**, peptide 32) was about 10 times more potent than the single mutant for inhibition of DNA binding. Giorello *et al.* adapted the H1-S6A, F8A peptide for cancer therapy by fusing it with penetratin [103]. They showed that this peptide inhibited MCF-7 cell growth over an 11-day experiment in which the cells were treated on days 1, 4 and 7. Furthermore, they demonstrated that the peptide induced apoptosis in the MCF-7 cells, and they confirmed that inhibition was due to prevention of the c-Myc/Max interaction and subsequent inhibition of transcriptional activation by c-Myc/Max. In an attempt to improve the stability and potency of the peptide, the group generated a retro-inverso version [104]. They found that the RI form of the penetratin-fused H1-S6A, F8A peptide was 30 – 35-fold more stable in cultured cells and 5 – 10-fold more potent than the native L-peptide in the 11-day MCF-7 proliferation experiment, and they confirmed these results in the HCT-116 colon caner cell line. The RI peptide was fairly stable *in vivo* when injected i.v. into nude mice, accumulating most efficiently in the liver and lung, and to a lesser extent in the spleen and kidney, and displayed a half-life of ~ 24 h [105]. However, so far, no tumor biodistribution or tumor reduction efficacy for this peptide has been published. In a recent report, the group identifies INI1, a component of the SWI/SNF complex, as an interacting partner for c-Myc, and concludes that the H1-S6A, F8A peptide could also be functioning by inhibiting this interaction [106].

The authors' lab is interested in the development of a polypeptide-based targetable macromolecular carrier for therapeutic peptides. The work was begun using the c-Myc H1-S6A, F8A peptide as a model TP. The polypeptide carrier used by the lab is elastin-like polypeptide (ELP), which is a 60 kDa polypeptide composed of repeated units of a 5 amino acid motif, VPGXG, where X can be any amino acid except proline [107]. ELP is ideal for use as a carrier for TPs because it is genetically encoded and can be expressed in *Escherichia coli*. Therefore, the codons coding for any TP of interest can be fused in frame to the ELP coding sequence, and the resulting chimeric polypeptide can be expressed and purified recombinantly. Also, ELP is attractive as a drug delivery vector because it is a macromolecule, which gives it a long plasma half-life and enhanced tumor targeting properties, and because it is thermally responsive. ELP reversibly forms aggregates in response to mild hyperthermia, so systemically circulating ELP can be targeted to a tumor site by applying mild hyperthermia at the site, which will cause the polypeptide to aggregate and accumulate there [108-110]. When the penetratin peptide was fused to the ELP N terminus and the c-Myc H1-S6A, F8A peptide to the ELP C terminus, it was shown that the resulting polypeptide (Pen-ELP-H1) could inhibit the proliferation of MCF-7 cells, and the inhibition was achieved by a single 1 h exposure to the polypeptide [111]. Furthermore, the intracellular delivery of Pen-ELP-H1 and the proliferation inhibition was enhanced when cells were treated for 1 h with hyperthermia (42°C). It was confirmed that Pen-ELP-H1 functioned by binding and sequestering newly translated c-Myc protein to the cytoplasm, thereby preventing its interaction with Max and blocking activation of c-Myc/Max target genes. Pen-ELP-H1 was also able to enhance the potency of the small molecule topoisomerase II inhibitors doxorubicin and etoposide in MCF-7, HeLa and MES-SA (uterine sarcoma) cells [112]. More recently, the authors have also evaluated two other ELP-H1 fusion polypeptides that contained the Tat or Bac CPPs at the N terminus, and determined that Bac-ELP-H1 was far more potent than the original Pen-ELP-H1 polypeptide owing to the fact that the Bac CPP directed nuclear localization of the molecule, where it could probably interfere directly with the c-Myc/Max or c-Myc/INI1 interaction [113]. The authors are now evaluating the lead Bac-ELP-H1 polypeptide in rodent models of breast cancer and glioblastoma, with promising results (unpublished data).

3. Conclusion

The work described in this review highlights the versatility and potential of TPs. The reviewed literature demonstrates that TPs can be developed for inhibition or reactivation of a huge variety of important signaling molecules. Furthermore, these peptides can be very specific for their target proteins and, in some cases, can also be specific for cancerous cell types. As knowledge grows about the proteins involved in tumor cell development, peptides will be the first available inhibitors for therapy of the newly discovered target proteins. Therefore, owing to their ease of design and production and wide spectrum of potential targets, TPs have a promising future in cancer therapy.

4. Expert opinion

Therapeutic peptides have many attributes that make them attractive as drugs for cancer therapy. First, because so much is known about the sequence and structure of interacting proteins, rational design of TPs to inhibit interactions of interest is relatively easy, and certainly much easier than designing small molecules to inhibit the same interactions. This gives drug developers access to inhibitors of many important protein–protein interactions to which small molecule inhibitors are not available. Furthermore, TPs can be very specific for their target protein, reducing the likelihood of off-target effects. However, so far, peptides have been largely ignored as potential therapies for cancer because of

their poor performance pharmacologically. Limitations of stability in the plasma, bioavailability and tumor cell penetration have prevented the advance of peptides beyond preclinical testing. Therefore, the key issue for development of this new class of drugs is not finding new TPs, but finding new ways to deliver TPs. What is needed most are carriers for these peptides that are capable of stabilizing them against proteases and delivering them out of the vascular space and into the tumor cells, where they can interact with their target proteins.

As discussed in this review, the limitation of peptide degradation in serum can be overcome by modifying peptides with non-natural amino acids or other chemical modifications, but these attempts only partially address the limitations of therapeutic peptides. Even if stabilized from proteases, peptides are still low molecular mass and charged, and rapid renal clearance, poor tumor penetration and immunogenicity are still drawbacks. A better solution might be to use macromolecular carriers to mediate peptide delivery. For example, research has shown that the plasma half-life of a small protein, the 20 kDa soybean trypsin inhibitor, can be increased ~ 10-fold by increasing its molecular mass to 127 kDa by fusing it to a dextran [114]. Elegant work by Dreher *et al*. using fluorescent dextrans demonstrated that, as molecular mass increases, the plasma half-life increases, but, at the same time, the rate of diffusion from the vascular space into the intratumoral space decreases [115]. The authors demonstrated that, for the dextrans used, a molecular mass of 40 – 70 kDa was the optimal size for maximizing both plasma half-life and tumor extravasation. Similar studies can be done for other types of polymeric carriers to determine their optimal molecular mass. Other benefits of macromolecular conjugation include lowered immunogenicity and passive accumulation in tumors owing to the enhanced permeability and retention (EPR) effect (reviewed in [116]). Many types of macromolecule have been used as drug carriers, most notably poly(ethylene glycol) (PEG) and *N*-(2-hydroxypropyl)methacrylamide (HPMA) [117,118], and all are capable of passive targeting by means of the EPR effect. However, the strategy of using macromolecular carriers can be improved further by using actively targeted high-molecular-mass carriers. Examples include antibody-fused macromolecules, targeted liposomes and stimulus responsive molecules (magnetic nanoparticles; thermal, pH, or ultrasound-sensitive hydrogels; and thermally responsive polypeptides [7,119,120]). Some work, including efforts by the authors' lab, is underway to apply macromolecular carriers for delivery of TPs, but more work is needed to demonstrate efficacy in preclinical models.

TPs, especially the signal transduction inhibitors discussed here, have a huge potential as chemotherapeutics for cancer as well as other diseases owing to their versatility. Using just the peptides discussed in this review, many potential target proteins can be treated for which no small molecule drugs exist; and, using the techniques reviewed here, many more peptides can be identified. Small molecule drug development has traditionally focused on finding inhibitors of enzymes, probably because it is easier to assay drug activity. Most of the current focus on development of targeted drugs is on small molecule kinase inhibitors. As shown here, peptides can be just as effective for inhibition of protein–protein interactions between non-enzymatic proteins as they are of inhibiting enzymes. Therefore, the potential for development of targeted inhibitors for any important intraprotein interaction is realized using peptide therapy and, if the drug delivery hurdles can be overcome, there is no limit to the possible applications of peptide therapeutics.

Development of TPs will become especially important as the age of personalized medicine approaches. In the field of diagnostic medicine, the goal of the near future is to be able to examine a patient's tumor individually, determine which oncogenic transformations are present, and tailor the therapy towards the specific aberrant proteins. One can envisage having an arsenal of peptides available to inhibit many known oncogenic interactions and, as personal diagnostic technology grows, being able to apply a specific peptide or cocktail of peptides to a patient based on the etiology of his or her cancer type. For this vision to become reality, cancer researchers need to continue to identify aberrantly expressed or mutated proteins that lead to tumorigenesis, discover peptides capable of modulating these proteins, and develop an effective and practical means of delivering the peptide therapeutics to the tumor in the clinical setting.

Declaration of interest

D Raucher is President of Thermally Targeted Therapeutics (TTT), a private company working to commercialize ELP drug delivery technology. GL Bidwell is a paid consultant of TTT.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Talmadge JE. Pharmacodynamic aspects of peptide administration biological response modifiers. Adv Drug Deliv Rev 1998;33(3):241-52

• **This work describes the current limitations of *in vivo* peptide delivery.**

2. Lipka E, Crison J, Amidon GL. Transmembrane transport of peptide type compounds: prospects for oral delivery. J Control Release 1996;39(2-3):121-9
3. Futaki S, Suzuki T, Ohashi W, et al. Arginine-rich peptides. An abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery. J Biol Chem 2001;276(8):5836-40
4. Massodi I, Bidwell GL 3rd, Raucher D. Evaluation of cell penetrating peptides fused to elastin-like polypeptide for drug delivery. J Control Release 2005;108(2-3):396-408
5. Vives E, Richard JP, Rispal C, et al. TAT peptide internalization: seeking the mechanism of entry. Curr Protein Pept Sci 2003;4(2):125-32
6. Vives E. Cellular uptake [correction of utake] of the Tat peptide: an endocytosis mechanism following ionic interactions. J Mol Recognit 2003;16(5):265-71
7. Raucher D, Massodi I, Bidwell GL. Thermally targeted delivery of chemotherapeutics and anti-cancer peptides by elastin-like polypeptide. Expert Opin Drug Deliv 2008;5(3):353-69
8. Torchilin VP. Tat peptide-mediated intracellular delivery of pharmaceutical nanocarriers. Adv Drug Deliv Rev 2008;60(4-5):548-58
9. Derossi D, Joliot AH, Chassaing G, et al. The third helix of the Antennapedia homeodomain translocates through biological membranes. J Biol Chem 1994;269(14):10444-50
10. Vives E, Brodin P, Lebleu B. A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. J Biol Chem 1997;272(25):16010-7
11. Lin YZ, Yao SY, Veach RA, et al. Inhibition of nuclear translocation of transcription factor NF-kappa B by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence. J Biol Chem 1995;270(24):14255-8
12. Sadler K, Eom KD, Yang JL, et al. Translocating proline-rich peptides from the antimicrobial peptide bactenecin 7. Biochemistry 2002;41(48):14150-7
13. Derossi D, Chassaing G, Prochiantz A. Trojan peptides: the penetratin system for intracellular delivery. Trends Cell Biol 1998;8(2):84-7
14. Lundberg P, Langel U. A brief introduction to cell-penetrating peptides. J Mol Recognit 2003;16(5):227-33
15. Gupta B, Levchenko TS, Torchilin VP. Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides. Adv Drug Deliv Rev 2005;57(4):637-51
16. Barbacid M. Ras genes. Annu Rev Biochem 1987;56:779-827
17. Feig LA. The many roads that lead to Ras. Science 1993;260(5109):767-8
18. Adari H, Lowy DR, Willumsen BM, et al. Guanosine triphosphatase activating protein (GAP) interacts with the p21 ras effector binding domain. Science 1988;240(4851):518-21
19. Moodie SA, Willumsen BM, Weber MJ, et al. Complexes of Ras.GTP with Raf-1 and mitogen-activated protein kinase kinase. Science 1993;260(5114):1658-61
20. Barbacid M. Involvement of ras oncogenes in the initiation of carcinogen-induced tumors. Princess Takamatsu Symp 1986;17:43-53
21. Chung DL, Brandt-Rauf P, Murphy RB, et al. A peptide from the GAP-binding domain of the ras-p21 protein and azatyrosine block ras-induced maturation of Xenopus oocytes. Anticancer Res 1991;11(4):1373-8

•• **This paper describes one of the first TPs developed, aimed at inhibiting Ras signaling.**

22. Haspel J, Dykes DC, Friedman FK, et al. Inhibition of ras-oncogene-encoded p21 protein-induced maturation of oocytes by p21 peptide sequences predicted to be effector domain sites by molecular modeling. Med Sci Res 1992;20:809-11
23. Adler V, Pincus MR, Brandt-Rauf PW, et al. Complexes of p21RAS with JUN N-terminal kinase and JUN proteins. Proc Natl Acad Sci USA 1995;92(23):10585-9
24. Chie L, Chen JM, Friedman FK, et al. Identification of the site of inhibition of oncogenic ras-p21-induced signal transduction by a peptide from a ras effector domain. J Protein Chem 1999;18(8):881-4
25. Barnard D, Sun H, Baker L, et al. In vitro inhibition of Ras-Raf association by short peptides. Biochem Biophys Res Commun 1998;247(1):176-80
26. Kanovsky M, Michl J, Botzolaki G, et al. Peptides designed from molecular modeling studies of the ras-p21 protein induce phenotypic reversion of a pancreatic carcinoma cell line but have no effect on normal pancreatic acinar cell growth. Cancer Chemother Pharmacol 2003;52(3):202-8
27. Adler V, Bowne W, Kamran I, et al. Two peptides derived from ras-p21 induce either phenotypic reversion or tumor cell necrosis of ras-transformed human cancer cells. Cancer Chemother Pharmacol 2008;62(3):491-8
28. Chen JM, Friedman FK, Hyde MJ, et al. Molecular dynamics analysis of the structures of ras-guanine nucleotide exchange protein (SOS) bound to wild-type and oncogenic ras-p21. Identification of effector domains of SOS. J Protein Chem 1999;18(8):867-74
29. Chie L, Chen JM, Friedman FK, et al. Inhibition of oncogenic and activated wild-type ras-p21 protein-induced oocyte maturation by peptides from the guanine-nucleotide exchange protein, SOS, identified from molecular dynamics calculations. Selective inhibition of oncogenic ras-p21. J Protein Chem 1999;18(8):875-9
30. Chen JM, Friedman FK, Brandt-Rauf PW, et al. Comparison of the average structures, from molecular dynamics, of complexes of GTPase activating protein (GAP) with oncogenic and wild-type ras-p21: identification of potential effector domains. J Protein Chem 2002;21(5):349-59
31. Friedman FK, Chie L, Chung D, et al. Inhibition of ras-induced oocyte maturation by peptides from ras-p21 and GTPase activating protein (GAP) identified as being effector domains from molecular dynamics calculations. J Protein Chem 2002;21(5):361-6
32. Chen JM, Monaco R, Manolatos S, et al. Molecular dynamics on complexes of ras-p21 and its inhibitor protein, rap-1A,

bound to the ras-binding domain of the raf-p74 protein: identification of effector domains in the raf protein. J Protein Chem 1997;16(6):619-29

33. Chung D, Amar S, Glozman A, et al. Inhibition of oncogenic and activated wild-type ras-p21 protein-induced oocyte maturation by peptides from the ras-binding domain of the raf-p74 protein, identified from molecular dynamics calculations. J Protein Chem 1997;16(6):631-5
34. Clark GJ, Drugan JK, Terrell RS, et al. Peptides containing a consensus Ras binding sequence from Raf-1 and the GTPase activating protein NF1 inhibit Ras function. Proc Natl Acad Sci USA 1996;93(4):1577-81
35. Rojas M, Yao S, Lin YZ. Controlling epidermal growth factor (EGF)-stimulated Ras activation in intact cells by a cell-permeable peptide mimicking phosphorylated EGF receptor. J Biol Chem 1996;271(44):27456-61
36. Buday L, Downward J. Epidermal growth factor regulates p21ras through the formation of a complex of receptor, Grb2 adapter protein, and Sos nucleotide exchange factor. Cell 1993;73(3):611-20
37. Rojas M, Yao S, Donahue JP, et al. An alternative to phosphotyrosine-containing motifs for binding to an SH2 domain. Biochem Biophys Res Commun 1997;234(3):675-80
38. Qian Y, Blaskovich MA, Saleem M, et al. Design and structural requirements of potent peptidomimetic inhibitors of p21ras farnesyltransferase. J Biol Chem 1994;269(17):12410-3
39. Brown MS, Goldstein JL, Paris KJ, et al. Tetrapeptide inhibitors of protein farnesyltransferase: amino-terminal substitution in phenylalanine-containing tetrapeptides restores farnesylation. Proc Natl Acad Sci USA 1992;89(17):8313-6
40. Reiss Y, Goldstein JL, Seabra MC, et al. Inhibition of purified p21ras farnesyl:protein transferase by Cys-AAX tetrapeptides. Cell 1990;62(1):81-8
41. Ohkanda J, Knowles DB, Blaskovich MA, et al. Inhibitors of protein farnesyltransferase as novel anticancer agents. Curr Top Med Chem 2002;2(3):303-23
42. Konstantinopoulos PA, Karamouzis MV, Papavassiliou AG. Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. Nat Rev Drug Discov 2007;6(7):541-55
43. Sousa SF, Fernandes PA, Ramos MJ. Farnesyltransferase inhibitors: a detailed chemical view on an elusive biological problem. Curr Med Chem 2008;15(15):1478-92
44. Cobb MH, Hepler JE, Cheng M, et al. The mitogen-activated protein kinases, ERK1 and ERK2. Semin Cancer Biol 1994;5(4):261-8
45. Treisman R. Regulation of transcription by MAP kinase cascades. Curr Opin Cell Biol 1996;8(2):205-15
46. Adler V, Qu Y, Smith SJ, et al. Functional interactions of Raf and MEK with Jun-N-terminal kinase (JNK) result in a positive feedback loop on the oncogenic Ras signaling pathway. Biochemistry 2005;44(32):10784-95
47. Kelemen BR, Hsiao K, Goueli SA. Selective in vivo inhibition of mitogen-activated protein kinase activation using cell-permeable peptides. J Biol Chem 2002;277(10):8741-8
48. Adler V, Yin Z, Fuchs SY, et al. Regulation of JNK signaling by GSTp. Embo J 1999;18(5):1321-34
49. Adler V, Pincus MR. Effector peptides from glutathione-S-transferase-pi affect the activation of jun by jun-N-terminal kinase. Ann Clin Lab Sci 2004;34(1):35-46
50. Chie L, Adler V, Friedman FK, et al. An effector peptide from glutathione-S-transferase-pi strongly and selectively blocks mitotic signaling by oncogenic ras-p21. Protein J 2004;23(3):235-8
51. Barr RK, Kendrick TS, Bogoyevitch MA. Identification of the critical features of a small peptide inhibitor of JNK activity. J Biol Chem 2002;277(13):10987-97

• **This paper describes a peptide-based strategy for inhibiting the JNK signaling cascade.**

52. Bonny C, Oberson A, Negri S, et al. Cell-permeable peptide inhibitors of JNK: novel blockers of beta-cell death. Diabetes 2001;50(1):77-82
53. Borsello T, Clarke PG, Hirt L, et al. A peptide inhibitor of c-Jun N-terminal kinase protects against excitotoxicity and cerebral ischemia. Nat Med 2003;9(9):1180-6
54. Bogoyevitch MA. Therapeutic promise of JNK ATP-noncompetitive inhibitors. Trends Mol Med 2005;11(5):232-9
55. Bogoyevitch MA, Barr RK, Ketterman AJ. Peptide inhibitors of protein kinases-discovery, characterisation and use. Biochim Biophys Acta 2005;1754(1-2):79-99

•• **This review covers the development of kinase-inhibiting peptides, including some less extensively studied peptides not covered in this review owing to lack of space.**

56. Bogoyevitch MA, Arthur PG. Inhibitors of c-Jun N-terminal kinases: JuNK no more? Biochim Biophys Acta 2008;1784(1):76-93
57. Gao YJ, Cheng JK, Zeng Q, et al. Selective inhibition of JNK with a peptide inhibitor attenuates pain hypersensitivity and tumor growth in a mouse skin cancer pain model. Exp Neurol 2009. In press

• **This paper describes an *in vivo* application of the NBD peptide for treatment of melanoma.**

58. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. Cell 2008;132(3):344-62
59. May MJ, D'Acquisto F, Madge LA, et al. Selective inhibition of NF-kappaB activation by a peptide that blocks the interaction of NEMO with the IkappaB kinase complex. Science 2000;289(5484):1550-4

• **This paper is one of the first descriptions of an NF-κB modulating peptide, and the peptide described here was later used effectively in animal models of many diseases with an inflammatory component, and in an animal model of melanoma.**

60. Dasgupta S, Jana M, Zhou Y, et al. Antineuroinflammatory effect of NF-kappaB essential modifier-binding domain peptides in the adoptive transfer model of experimental allergic encephalomyelitis. J Immunol 2004;173(2):1344-54
61. di Meglio P, Ianaro A, Ghosh S. Amelioration of acute inflammation by systemic administration of a cell-permeable peptide inhibitor of NF-kappaB activation. Arthritis Rheum 2005;52(3):951-8
62. Tas SW, Vervoordeldonk MJ, Hajji N, et al. Local treatment with the selective IkappaB kinase beta inhibitor NEMO-binding domain peptide ameliorates synovial

inflammation. Arthritis Res Ther 2006;8(4):R86

63. Ghosh A, Roy A, Liu X, et al. Selective inhibition of NF-kappaB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease. Proc Natl Acad Sci USA 2007;104(47):18754-9

64. Rehman KK, Bertera S, Bottino R, et al. Protection of islets by in situ peptide-mediated transduction of the Ikappa B kinase inhibitor Nemo-binding domain peptide. J Biol Chem 2003;278(11):9862-8

65. Thomas RP, Farrow BJ, Kim S, et al. Selective targeting of the nuclear factor-kappaB pathway enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated pancreatic cancer cell death. Surgery 2002;132(2):127-34

66. Biswas DK, Shi Q, Baily S, et al. NF-kappa B activation in human breast cancer specimens and its role in cell proliferation and apoptosis. Proc Natl Acad Sci USA 2004;101(27):10137-42

67. Tapia MA, Gonzalez-Navarrete I, Dalmases A, et al. Inhibition of the canonical IKK/NF kappa B pathway sensitizes human cancer cells to doxorubicin. Cell Cycle 2007;6(18):2284-92

68. Ianaro A, Tersigni M, Belardo G, et al. NEMO-binding domain peptide inhibits proliferation of human melanoma cells. Cancer Lett 2009;274(2):331-6

69. Agou F, Traincard F, Vinolo E, et al. The trimerization domain of NEMO is composed of the interacting C-terminal CC2 and LZ coiled-coil subdomains. J Biol Chem 2004;279(27):27861-9

70. Agou F, Courtois G, Chiaravalli J, et al. Inhibition of NF-kappa B activation by peptides targeting NF-kappa B essential modulator (nemo) oligomerization. J Biol Chem 2004;279(52):54248-57

71. Carvalho G, Fabre C, Braun T, et al. Inhibition of NEMO, the regulatory subunit of the IKK complex, induces apoptosis in high-risk myelodysplastic syndrome and acute myeloid leukemia. Oncogene 2007;26(16):2299-307

72. Torgerson TR, Colosia AD, Donahue JP, et al. Regulation of NF-kappa B, AP-1, NFAT, and STAT1 nuclear import in T lymphocytes by noninvasive delivery of peptide carrying the nuclear localization sequence of NF-kappa B p50. J Immunol 1998;161(11):6084-92

- **This paper describes an NLS peptide capable of inhibiting nuclear localization of several important proteins, and this peptide has also been applied successfully in several animal models of inflammatory diseases.**

73. Letoha T, Somlai C, Takacs T, et al. A nuclear import inhibitory peptide ameliorates the severity of cholecystokinin-induced acute pancreatitis. World J Gastroenterol 2005;11(7):990-9

74. Saika S, Miyamoto T, Yamanaka O, et al. Therapeutic effect of topical administration of SN50, an inhibitor of nuclear factor-kappaB, in treatment of corneal alkali burns in mice. Am J Pathol 2005;166(5):1393-403

75. Gonzalez-Ramos R, Van Langendonckt A, Defrere S, et al. Agents blocking the nuclear factor-kappaB pathway are effective inhibitors of endometriosis in an in vivo experimental model. Gynecol Obstet Invest 2008;65(3):174-86

76. Poulaki V, Mitsiades CS, Joussen AM, et al. Constitutive nuclear factor-kappaB activity is crucial for human retinoblastoma cell viability. Am J Pathol 2002;161(6):2229-40

77. Starenki D, Namba H, Saenko V, et al. Inhibition of nuclear factor-kappaB cascade potentiates the effect of a combination treatment of anaplastic thyroid cancer cells. J Clin Endocrinol Metab 2004;89(1):410-8

78. Xu Y, Fang F, St Clair DK, et al. SN52, a novel nuclear factor-kappaB inhibitor, blocks nuclear import of RelB:p52 dimer and sensitizes prostate cancer cells to ionizing radiation. Mol Cancer Ther 2008;7(8):2367-76

79. Yan Liu X, Robinson D, Veach RA, et al. Peptide-directed suppression of a pro-inflammatory cytokine response. J Biol Chem 2000;275(22):16774-8

80. Fujihara SM, Cleaveland JS, Grosmaire LS, et al. A D-amino acid peptide inhibitor of NF-kappa B nuclear localization is efficacious in models of inflammatory disease. J Immunol 2000;165(2):1004-12

81. Yaron A, Gonen H, Alkalay I, et al. Inhibition of NF-kappa-B cellular function via specific targeting of the I-kappa-B-ubiquitin ligase. Embo J 1997;16(21):6486-94

82. Takada Y, Singh S, Aggarwal BB. Identification of a p65 peptide that selectively inhibits NF-kappa B activation induced by various inflammatory stimuli and its role in down-regulation of NF-kappaB-mediated gene expression and up-regulation of apoptosis. J Biol Chem 2004;279(15):15096-104

83. Hupp TR, Sparks A, Lane DP. Small peptides activate the latent sequence-specific DNA binding function of p53. Cell 1995;83(2):237-45

- **This paper describes the first peptides capable of restoring p53 function.**

84. Selivanova G, Iotsova V, Okan I, et al. Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. Nat Med 1997;3(6):632-8

85. Selivanova G, Ryabchenko L, Jansson E, et al. Reactivation of mutant p53 through interaction of a C-terminal peptide with the core domain. Mol Cell Biol 1999;19(5):3395-402

86. Kim AL, Raffo AJ, Brandt-Rauf PW, et al. Conformational and molecular basis for induction of apoptosis by a p53 C-terminal peptide in human cancer cells. J Biol Chem 1999;274(49):34924-31

87. Li Y, Rosal RV, Brandt-Rauf PW, et al. Correlation between hydrophobic properties and efficiency of carrier-mediated membrane transduction and apoptosis of a p53 C-terminal peptide. Biochem Biophys Res Commun 2002;298(3):439-49

88. Li Y, Mao Y, Rosal RV, et al. Selective induction of apoptosis through the FADD/caspase-8 pathway by a p53 c-terminal peptide in human pre-malignant and malignant cells. Int J Cancer 2005;115(1):55-64

89. Friedler A, Hansson LO, Veprintsev DB, et al. A peptide that binds and stabilizes p53 core domain: chaperone strategy for rescue of oncogenic mutants. Proc Natl Acad Sci USA 2002;99(2):937-42

90. Issaeva N, Friedler A, Bozko P, et al. Rescue of mutants of the tumor suppressor p53 in cancer cells by a designed peptide. Proc Natl Acad Sci USA 2003;100(23):13303-7

91. Böttger A, Bottger V, Sparks A, et al. Design of a synthetic Mdm2-binding mini

protein that activates the p53 response in vivo. Curr Biol 1997;7(11):860-9

•• **This paper describes a strategy for increasing peptide stability by expressing it as part of a mini protein.**

92. Wasylyk C, Salvi R, Argentini M, et al. p53 mediated death of cells overexpressing MDM2 by an inhibitor of MDM2 interaction with p53. Oncogene 1999;18(11):1921-34

93. Kanovsky M, Raffo A, Drew L, et al. Peptides from the amino terminal mdm-2-binding domain of p53, designed from conformational analysis, are selectively cytotoxic to transformed cells. Proc Natl Acad Sci USA 2001;98(22):12438-43

94. Do TN, Rosal RV, Drew L, et al. Preferential induction of necrosis in human breast cancer cells by a p53 peptide derived from the MDM2 binding site. Oncogene 2003;22(10):1431-44

95. Bowne WB, Sookraj KA, Vishnevetsky M, et al. The penetratin sequence in the anticancer PNC-28 peptide causes tumor cell necrosis rather than apoptosis of human pancreatic cancer cells. Ann Surg Oncol 2008;15(12):3588-600

96. Michl J, Scharf B, Schmidt A, et al. PNC-28, a p53-derived peptide that is cytotoxic to cancer cells, blocks pancreatic cancer cell growth in vivo. Int J Cancer 2006;119(7):1577-85

•• **This paper describes the application of a p53-derived peptide in a mouse xenograft model, and this work is some of the most advanced preclinical testing published with a TP in a cancer model.**

97. Harbour JW, Worley L, Ma D, et al. Transducible peptide therapy for uveal melanoma and retinoblastoma. Arch Ophthalmol 2002;120(10):1341-6

98. Midgley CA, Desterro JM, Saville MK, et al. An N-terminal p14ARF peptide blocks Mdm2-dependent ubiquitination in vitro and can activate p53 in vivo. Oncogene 2000;19(19):2312-23

99. Karlsson GB, Jensen A, Stevenson LF, et al. Activation of p53 by scaffold-stabilised expression of Mdm2-binding peptides: visualisation of reporter gene induction at the single-cell level. Br J Cancer 2004;91(8):1488-94

100. Pelengaris S, Khan M, Evan G. c-MYC: more than just a matter of life and death. Nat Rev Cancer 2002;2(10):764-76

101. Blackwood EM, Luscher B, Eisenman RN. Myc and Max associate in vivo. Genes Dev 1992;6(1):71-80

102. Draeger LJ, Mullen GP. Interaction of the bHLH-zip domain of c-Myc with H1-type peptides. Characterization of helicity in the H1 peptides by NMR. J Biol Chem 1994;269(3):1785-93

• **This paper introduces the only peptide described so far capable of inhbiting c-Myc function.**

103. Giorello L, Clerico L, Pescarolo MP, et al. Inhibition of cancer cell growth and c-Myc transcriptional activity by a c-Myc helix 1-type peptide fused to an internalization sequence. Cancer Res 1998;58(16):3654-9

104. Pescarolo MP, Bagnasco L, Malacarne D, et al. A retro-inverso peptide homologous to helix 1 of c-Myc is a potent and specific inhibitor of proliferation in different cellular systems. Faseb J 2001;15(1):31-3

105. Nieddu E, Melchiori A, Pescarolo MP, et al. Sequence specific peptidomimetic molecules inhibitors of a protein-protein interaction at the helix 1 level of c-Myc. Faseb J 2005;19(6):632-4

106. Bagnasco L, Tortolina L, Biasotti B, et al. Inhibition of a protein-protein interaction between INI1 and c-Myc by small peptidomimetic molecules inspired by Helix-1 of c-Myc: identification of a new target of potential antineoplastic interest. Faseb J 2007;21(4):1256-63

107. Urry DW, Luan C-H, Parker TM, et al. Temperature of polypeptide inverse temperature transition depends on mean residue hydrophobicity. J Am Chem Soc 1991;113:4346-8

108. Meyer DE, Kong GA, Dewhirst MW, et al. Targeting a genetically engineered elastin-like polypeptide to solid tumors by local hyperthermia. Cancer Res 2001;61(4):1548-54

109. Liu W, Dreher MR, Furgeson DY, et al. Tumor accumulation, degradation and pharmacokinetics of elastin-like polypeptides in nude mice. J Control Release 2006;116(2):170-8

110. Dreher MR, Liu W, Michelich CR, et al. Thermal cycling enhances the accumulation of a temperature-sensitive biopolymer in solid tumors. Cancer Res 2007;67(9):4418-24

111. Bidwell GL 3rd, Raucher D. Application of thermally responsive polypeptides directed against c-Myc transcriptional function for cancer therapy. Mol Cancer Ther 2005;4(7):1076-85

•• **This work demonstrates the potential of using a targeted carrier for delivery of a TP.**

112. Bidwell GL 3rd, Raucher D. Enhancing the antiproliferative effect of topoisomerase II inhibitors using a polypeptide inhibitor of c-Myc. Biochem Pharmacol 2006;71(3):248-56

113. Bidwell GL 3rd, Davis AN, Raucher D. Targeting a c-Myc inhibitory polypeptide to specific intracellular compartments using cell penetrating peptides. J Control Release 2009;135(1):2-10

114. Takakura Y, Fujita T, Hashida M, et al. Control of pharmaceutical properties of soybean trypsin inhibitor by conjugation with dextran. II: biopharmaceutical and pharmacological properties. J Pharm Sci 1989;78(3):219-22

115. Dreher MR, Liu W, Michelich CR, et al. Tumor vascular permeability, accumulation, and penetration of macromolecular drug carriers. J Natl Cancer Inst 2006;98(5):335-44

116. Torchilin VP, Lukyanov AN. Peptide and protein drug delivery to and into tumors: challenges and solutions. Drug Discov Today 2003;8(6):259-66

117. Kratz F, Abu Ajaj K, Warnecke A. Anticancer carrier-linked prodrugs in clinical trials. Expert Opin Investig Drugs 2007;16(7):1037-58

118. Bailon P, Won CY. PEG-modified biopharmaceuticals. Expert Opin Drug Deliv 2009;6(1):1-16

119. Soppimath KS, Aminabhavi TM, Dave AM, et al. Stimulus-responsive 'smart' hydrogels as novel drug delivery systems. Drug Dev Ind Pharm 2002;28(8):957-74

120. Yuan Q, Venkatasubramanian R, Hein S, et al. A stimulus-responsive magnetic nanoparticle drug carrier: magnetite encapsulated by chitosan-grafted-copolymer. Acta Biomater 2008;4(4):1024-37

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