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Grb7-based molecular therapeutics in cancer

Stephanie C. Pero, Roger J. Daly and David N. Krag

Traditional anti-cancer drugs preferentially kill rapidly growing tumour cells rather than normal cells. However, most of these drugs have no preferential selection towards cancer cells and are taken up by the whole body, resulting in significant adverse side effects. Therapeutic molecules that could specifically inhibit undesirable phenotypes are an attractive way of eliminating cancer cells. There is a widespread effort to develop inhibitors against signal transduction molecules that play a key role in the proliferative, migratory and invasive properties of a cancer cell. Grb7 is an adaptor-type signalling protein that is recruited via its Src-homology 2 (SH2) domain to a variety of tyrosine kinases. Grb7 is overexpressed in breast, oesophageal and gastric cancers, and may contribute to the invasive potential of cancer cells. Molecular interactions involving Grb7 therefore provide attractive targets for therapeutic intervention.

The development of new therapeutic molecules to target cancer cells has accelerated dramatically in recent years, partly owing to the success of genomic research in identifying target proteins involved in key signalling pathways. In a wide variety of cancer cells, these lead targets have been shown to be present in altered forms or have different expression levels that ultimately play a role in cancer progression. Target-specific inhibitors are believed to have a greater chance of exerting efficacy without the systemic toxicity associated with traditional methods of cancer treatment (e.g. chemotherapy). Chemotherapeutic agents target rapidly dividing cells, and thus normal cells with high proliferation rates are also affected. Several molecular therapeutics that

Stephanie C. Pero
Postdoctoral Associate, Department of Surgery and the Vermont Cancer Center, University of Vermont School of Medicine, Given Medical Building, Burlington, VT 05405, USA. Tel: +1 802 656 0766; Fax: +1 802 656 5833; E-mail: Stephanie.Pero@uvm.edu

Roger J. Daly
Head of the Signal Transduction Group, Cancer Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, New South Wales, 2010, Australia. Tel: +61 2 9295 8333; Fax: +61 2 9295 8321; E-mail: r.daly@garvan.org.au

David N. Krag (corresponding author)
Professor of Surgery, Department of Surgery and the Vermont Cancer Center, University of Vermont School of Medicine, Given Medical Building, Burlington, VT 05405, USA. Tel: +1 802 656 5828; Fax: +1 802 656 5833; E-mail: David.Krag@uvm.edu

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target signal transduction pathways are currently under evaluation. Although the targets are found in normal cells, the drugs, in most cases, have been remarkably nontoxic compared with conventional treatments (Refs 1, 2, 3, 4). The theories of developing molecular therapeutics became reality when Herceptin (4D5; a humanised antibody that targets ErbB2) and Glivec (Gleevec, STI571, imatinibi; a signal transduction inhibitor that targets Bcr–Abl) were approved by the U.S. Food and Drug Administration (Refs 2, 5).

The focus of this review will be on developing inhibitors to adaptor proteins containing Src-homology 2 (SH2) domains, which mediate important signal transmission functions within cells. There are several SH2-domain-containing proteins being considered as possible targets for tumour-specific ligand-based therapy, including Grb2, ZAP-70 and p56^{lck} (Refs 6, 7, 8). Recently, the Grb7 molecule has also been identified as a possible target for therapeutic development (Ref. 9), and this will form the focus of this review.

The SH2 domain

The term SH2 was first coined by Pawson and colleagues when comparing the primary sequence of Fps and Src tyrosine kinases (Refs 10, 11). SH2 domains are approximately 100 amino acids in length and serve a crucial role by transmitting intracellular signals downstream of receptor tyrosine kinases (RTKs) and non-RTKs (Refs 10, 11). Many SH2 domains function by binding phosphotyrosine (pY)-containing sequences (Refs 12, 13, 14). Although there are SH2 domains that are able to bind to sites that are not tyrosine phosphorylated, such as the SH2 domain in SLAM-associated protein (SAP) (Ref. 15), the binding affinity increases approximately fivefold to a phosphorylated target (Ref. 12).

The sequence surrounding the pY residues determines the particular binding specificity of a given group of SH2-domain-containing proteins (Ref. 16). Indeed, SH2 domains have a conserved pocket that recognises pY and a more variable pocket that confers specificity. In general, the specificity of SH2-domain binding is determined by residues C-terminal to the pY residue (e.g. pY-hydrophilic-hydrophilic-I/P or pY-hydrophobic-X-hydrophobic; where X is any amino acid) (Ref. 16). However, groups of SH2-domain-containing proteins have also been identified that suggest the residues N-terminal to the pY residue might also play a role (Refs 17, 18). The SAP SH2 domain is unique because it has a large binding site that recognises residues that are both N-terminal and C-terminal to the tyrosine residue (Ref. 12). Therefore, the specific signalling pathway activated by a RTK is partially dependent on their available SH2-domain binding sites. The SH2 domain in Grb7 plays an important role in binding to many different RTKs (Table 1) and other tyrosine-phosphorylated proteins, and is described in further detail below.

Grb7

Structure

The Grb7 family comprises Grb7, Grb10 and Grb14, which all share significant sequence homology. Each member of the Grb7 family was identified using a technique known as CORT (for 'cloning of receptor targets'), an expression/cloning system that uses a tyrosine-phosphorylated receptor as a probe to screen an expression library for proteins that contain SH2 domains (Ref. 19). Grb7 was identified using CORT screening of a mouse embryo cDNA expression library with the tyrosine-phosphorylated C-terminus of the epidermal growth factor receptor (EGFR) (Ref. 20). All of the Grb7 family members consist of three regions: an N-terminal proline-rich region; a central GM (for 'Grb and MIG') region; and a C-terminal SH2 domain (Fig. 1). Grb2 has an SH2 domain and similar binding partners to that of Grb7; however, it is not part of the family because its other domains are different, and will not be discussed further in this review. For more background information on Grb2 and other Grb molecules, refer to Margolis et al. (Ref. 14).

The N-terminus of the Grb7 family shares the least homology. However, it does have a highly conserved motif (PS/AIPNPFPEL) flanked by two clusters of basic residues. In general, proline-rich regions are known for binding regulatory or effector molecules with SH3 domains (e.g. Src tyrosine kinase) or WW domains (e.g. Nedd4 E3 ubiquitin ligase), which can potentially bind overlapping sites (Refs 13, 21). For example, the proline-rich motif of Grb10 may provide a binding site for the SH3 domains of c-Abl, Grb2 or Fyn in vitro (Ref. 22). However, no proteins have yet been identified that bind to this 90 amino acid region of Grb7.

The central GM region, which is approximately 320 amino acids in length, shows significant amino acid homology between the Grb7 family

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Table 1. Tyrosine kinases bound by the Grb7 SH2 domain

Tyrosine kinase[a]	Function	Tyrosine kinase motif bound by Grb7 SH2[b]	Ref.
EGFR	Growth factor receptor	Unknown	19
ErbB2 (pY1139)	Growth factor receptor	PQPE **pY**VN QPD	25
ErbB3 (pY1180)	Growth factor receptor	DEEYE **pY**MN RRR	59
ErbB3 (pY1243)	Growth factor receptor	DEDYE **pY**MN RQR	59
ErbB4	Growth factor receptor	Unknown	59
Tek/Tie2 (pY1100)	Endothelial receptor tyrosine kinase	MLEERKT **pY**VN TTLYE	32
cKit/stem cell receptor (pY936)	Receptor tyrosine kinase	ASTNHI **pY**SN LANCS	76
FAK (pY397)	Cytoplasmic tyrosine kinase	SETDD **pY**AE IIDE	34
SHPTP-2 (pY580)	Protein tyrosine phosphatase	**pY**EN V	77
SHC (pY317)	Adaptor protein	**pY**VN V	25
PDGFRβ (pY716)	Growth factor receptor	RPPSAEL **pY**SN ALPVGVG	78
PDGFRβ (pY775)	Growth factor receptor	IESSNYMAP **pY**DN YVPSAPER	78
Ret	Receptor tyrosine kinase	Unknown	79
Insulin receptor	Receptor tyrosine kinase	Unknown	80
EphB1 (pY928)	Receptor tyrosine kinase	**pY**RD	81
Caveolin (pY14)	Phosphoprotein	GHL **pY**TV PI	82
RndI (pY74)	GTPase	YDN[c]	30

[a] The precise tyrosine residue of the Grb7 binding site on each target is given in parentheses.
[b] The phosphotyrosine residue (pY) is shown in bold within their precise binding motifs, many of which contain a YXN motif.
[c] It has been suggested that RndI binds the Grb7 SH2 domain in a pY-independent manner (Ref. 30).

Abbreviations: EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; PDGFR, platelet-derived growth factor receptor; SHPTP-2, SH2-containing protein tyrosine phosphatase; SH2, Src-homology 2.

and the *Caenorhabditis elegans* protein MIG-10 (Refs 23, 24, 25). Interestingly, MIG-10 is required for the long-range migration of neuronal cells during embryogenesis (Ref. 26). This conserved sequence homology suggests that Grb7 might also be involved in the migration process in mammalian cells. Within the GM region, additional regions have also been identified: the Pleckstrin homology (PH) domain, a putative RA (for 'Ral GDS/AF6' or 'Ras-associating') domain and a functional region called BPS (for 'between the PH and SH2 domains'). The PH domain of Grb7 binds to specific phosphoinositides (Ref. 27), which might allow PH-domain-containing proteins to respond to lipid messengers, for example by relocation to membranes. The function of the RA domain in Grb7 has not been identified, although its presence suggests that Grb7 might have a role in the Ras signalling pathway and be important for cell proliferation (Ref. 28). The practical importance of the RA domain is unknown. He et al. suggest that the sequence differences found in the BPS and SH2 domains of the Grb7 family members could be responsible for their specificity for tyrosine kinase receptors in different signalling pathways (Ref. 29).

The SH2 domain plays an important role in the binding of Grb7 to many different RTKs and other tyrosine-phosphorylated proteins. Table 1 lists identified target proteins and their precise binding motifs, many of which contain a YXN motif. One group has suggested that RndI binds the Grb7 SH2 domain in a pY-independent manner (Ref. 30). Although the Grb7 family exhibits significant sequence homology and a common overall structure, its members have different binding selectivities. For example, the SH2 domains of Grb14 and Grb7 share 67% amino acid identity yet the Grb7 SH2 domain binds strongly to ErbB2 and the Grb14 SH2 domain

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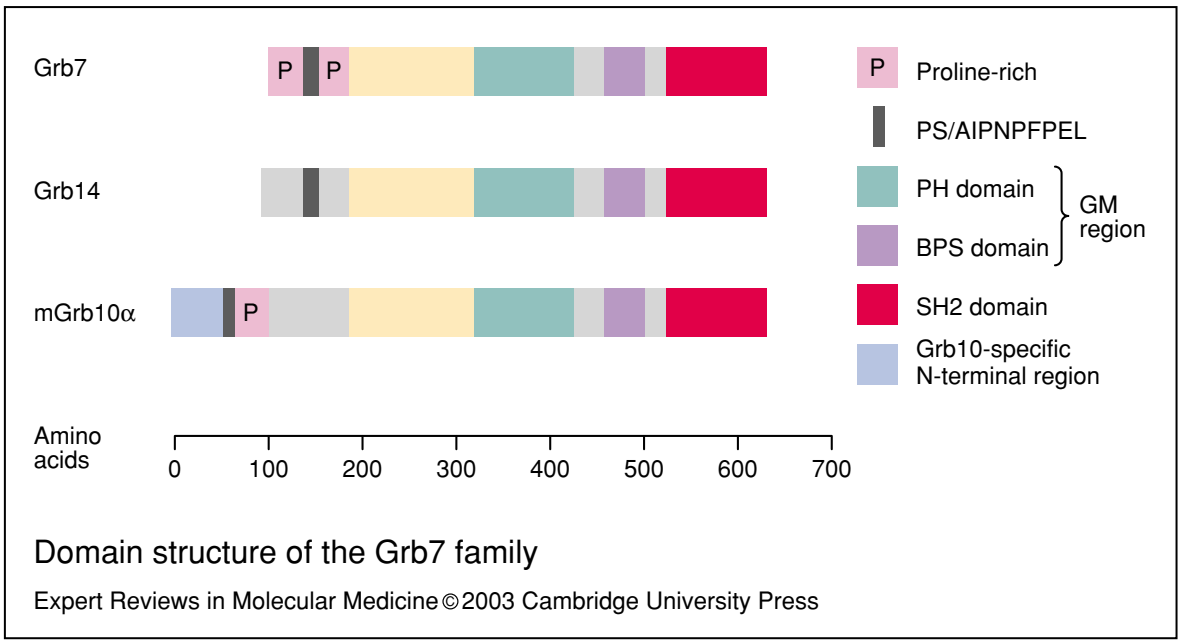

Figure 1. Domain structure of the Grb7 family. Functional domains and motifs are displayed for Grb7, Grb14 and mGrb10α. This figure depicts the N-terminal proline-rich region and PS/AIPNPFPEL motif, the GM region containing the Pleckstrin homology (PH) domain and BPS domains, and the Src-homology 2 (SH2) domain for each family member. The scale represents the size in amino acids. mGrb10α was the first of six isoforms identified for Grb10 and has a specific insert that precedes the RA domain (not shown). Figure adapted with thanks from a figure drawn by Rania Kairouz (PhD thesis, Garvan Institute of Medical Research, Sydney, Australia).

binds only very weakly (Ref. 31). It is now known that there are two important residues (βD5 and βD6) within the putative βD strand of the SH2 domain of Grb7 that are required to bind to ErbB2. These residues are Tyr, Leu in the Grb7 SH2 and Phe, Gln in the Grb14 SH2. Substituting individual amino acids in the Grb14 SH2 domain with the corresponding residues from Grb7 demonstrated that a Gln to Leu change at the βD6 position imparted high-affinity ErbB2 interaction, paralleled by a marked increase in affinity for the Tyr1139 phosphopeptide of ErbB2. Similarly, Grb14 βD5/βD6 residues were mutated to mimic the Grb7 βD5/βD6 residues and resulted in binding of Grb14 to ErbB2 phosphopeptides. Although Grb14 does not normally bind to ErbB2, suggesting different binding properties, this is not true for all of their target proteins. For example, Grb7 and Grb14 have been shown to be potential in vivo binding partners to Tek, an RTK restricted to endothelial cells (Ref. 32). However, the binding sites on Tek for Grb7 and Grb14 are different. This further demonstrates the binding selectivity of these SH2 domains.

Expression

In human tissues, Grb7 is expressed in the pancreas, kidney, placenta, prostate and small intestines (Ref. 22). By contrast, in mouse tissues, Grb7 expression is more limited, and is found only in kidney and liver (Ref. 19). Grb7 is localised in both the cytoplasm and focal contacts found on discrete regions of the plasma membrane (Ref. 33). The localisation to focal contacts can be eliminated by deleting the SH2 domain of Grb7, which suggests the importance of the SH2 domain for localisation. Recently, Shen et al. demonstrated that the PH domain mediates Grb7 binding to membrane phospholipids both in vitro and in intact cells (Ref. 27). Additional studies are required to gain a true understanding of Grb7 localisation.

Function?

Grb7 has been shown in numerous studies to interact with a variety of cell-surface receptors and other signalling molecules. Most of these interactions occur through the interaction of the SH2 domain of Grb7 and pY residues of activated signalling target proteins (Table 1), and it is

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likely that these proteins are upstream regulators of Grb7 function. Little is known about the downstream effectors of Grb7 and the precise role of this adaptor molecule. However, functional studies utilising cellular models have generated clues to help elucidate biological function, including in cell migration and cancer.

The role of Grb7 in cell migration was investigated after the identification of focal adhesion kinase (FAK) as a binding partner (Ref. 34). FAK is part of the tyrosine kinase family that activates integrin signal cascades regulating cell migration, survival and proliferation (Refs 35, 36, 37, 38, 39). Grb7 binds to the phosphorylated Tyr397 of FAK, which represents the major docking site for recruitment of Src, phospholipase Cγ1 (PLC-γ1) and phosphatidylinositol 3-kinase (PI3-kinase) (Refs 40, 41, 42, 43, 44, 45). In cells that overexpress FAK, Grb7 is localised to the focal contacts and is tyrosine phosphorylated through its cell-adhesion-dependent association with FAK (Refs 33, 34). Overexpression of Grb7 in fibroblasts enhances migration towards fibronectin, and overexpression of a dominant-negative Grb7 SH2 domain reduces fibroblast migration (Ref. 33). This study illustrates the crucial role Grb7 has in cell migration with no effect on cell-cycle progression. Moreover, expression of an antisense Grb7 RNA construct in oesophageal carcinoma cells markedly decreased EGF-stimulated cell migration without altering cell proliferation (Ref. 46).

The PH domain of Grb7 has been shown to interact with phosphoinositides in vitro and in intact cells. This interaction is important for mediating cell migration, which is enhanced by cell adhesion to fibronectin (Ref. 27). The phosphorylation of Grb7 at focal contacts, rather than its association with FAK, is necessary for stimulation of cell migration. In addition, Shen et al. (Ref. 27) demonstrated that Grb7 association with phosphoinositides was increased both by FAK binding to PI3-kinase via its pY397 residue and by integrin-mediated cell adhesion. These studies suggest that Grb7 is a downstream effector of PI3-kinase in the regulation of cell migration (Ref. 27).

Grb7 and cancer

Upregulation of Grb7 is found in a subset of breast, oesophageal and gastric cancers (Refs 25, 47, 48). Grb7 is an especially promising breast cancer target as it maps closely to the gene encoding ErbB2 on chromosome 17q and is co-amplified and overexpressed with ErbB2 in a subset of breast cancers (Ref. 25).

The importance of ErbB2 in breast cancer is well established: (1) ErbB2 is overexpressed on the tumour cells of approximately 30% of breast cancer patients and in more than half of ductal carcinoma in situ (an early form of breast cancer that is intraductal and non-invasive at this stage), which suggests that ErbB2 overexpression might be an important initiating event in breast cancer (Refs 49, 50); (2) ErbB2 expression is associated with poor prognosis (Refs 50, 51, 52); and (3) ErbB2 has low expression on most normal tissues (Refs 53, 54, 55). Furthermore, blocking the function of ErbB2 inhibits proliferation of cancer cells, as demonstrated in preclinical (Refs 56, 57) and clinical studies (Refs 2, 55).

A relatively recent study has demonstrated a relationship between Grb7 and the ErbB family of RTKs in human oesophageal and breast cancers. Co-expression of Grb7 with either EGFR or ErbB2 was detected in 31% of oesophageal carcinomas and was significantly correlated with extramucosal tumour invasion (Ref. 58). Interestingly, there was no amplification of the gene encoding Grb7 in oesophageal cancers but Grb7 mRNA was found to be overexpressed in 43.8% of oesophageal tumours examined (Ref. 58). In addition, at least 70% of the ErbB2-overexpressing breast tumours examined also overexpressed Grb7 (Ref. 25). Moreover, in a subgroup of human breast cancer cell lines, Grb7 associates and co-expresses with ErbB3 and ErbB4, which are known to heterodimerise with ErbB2 (Ref. 59).

The importance of Grb7 in tumour progression has been suggested by several studies (Refs 34, 46, 47, 58). In one recent study, histological expression of Grb7 in primary squamous cell oesophageal tumours at the time of surgical resection showed Grb7 to be overexpressed in 14 out of 31 oesophageal carcinomas as compared with adjacent normal mucosa. The overexpression of Grb7 in these histological sections was significantly correlated with the presence of lymph node metastases (Ref. 47). In addition, in an oesophageal cancer cell line, Grb7 was shown to be phosphorylated on tyrosine in response to EGF treatment or attachment of the cells to extracellular matrix proteins including fibronectin (Ref. 47).

Not only is Grb7 associated with tumour-related molecules, but it has also been demonstrated to have a direct role in cancer cell

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migration. The invasive role of Grb7 has also been demonstrated for its isoform, Grb7V. Grb7V is a novel splice variant lacking 88 bp in its C-terminus, resulting in a frameshift and a substitution of the SH2 domain for a short hydrophobic sequence. In an oesophageal cancer cell line, downregulation of both Grb7 and Grb7V using antisense technology inhibited the invasive properties of the cells in vitro (Ref. 46). Expression of Grb7V is restricted to invasive and metastatic oesophageal cancers, and increases in metastatic deposits in the lymph nodes. It is also proposed that Grb7V contributes to a more-invasive phenotype in oesophageal cancers, possibly as a result of its constitutive tyrosine phosphorylation (Ref. 46). These findings support the idea that Grb7 and Grb7V are involved in cell invasion and metastatic progression of human oesophageal cancer.

Development of Grb7-targeted therapeutics

A tremendous research effort in the field of drug discovery has been placed on the development of small-molecule and peptidomimetic inhibitors to signal transduction molecules (Refs 6, 7, 8). The development of SH2 antagonists of Grb2, ZAP-70 and $p56^{lck}$ has validated the idea that SH2-specific inhibitors can effectively inhibit the phenotypes associated with disease (Refs 6, 60). For example, many different Grb2 inhibitors that target the SH2 domain are being developed as cancer therapeutic agents (Refs 53, 54, 55, 61, 62). One molecule based on the pYIN peptide, which is one of the shortest peptide sequences retaining micromolar affinity for Grb2 SH2 (Ref. 63), led to the development of CGP78850. This small-molecule inhibitor blocks EGFR–Grb2 and Shc–Grb2 binding in living cells, and inhibits growth in RTK-transformed cell lines (Ref. 55). In addition, CGP78850 and a pro-drug derivative, referred to as CGP85793, are able to inhibit cell motility in A431 cells (Ref. 64).

As discussed above, Grb7 shows several promising features as a potential therapeutic target in tumour cells. These features include its overexpression in breast, oesophageal and gastric cancers; its association with tumour-related molecules; its role in cancer cell migration; and its limited tissue distribution. In particular, its limited tissue distribution is unlike that of many other SH2-domain-containing proteins, which are usually ubiquitously expressed (Ref. 14), and is a distinct advantage for targeting therapeutics to cancer cells. Grb7-specific inhibitors are in the early stages of development. Peptide inhibitors have recently been identified that bind to the SH2 domain of Grb7 and inhibit the association of Grb7 with the family of ErbB RTKs (Ref. 9). Importantly, there was no detectable binding to the SH2 domain of the closely related family member Grb14 or the SH2 domain of Grb2, which exhibits an overlapping binding selectivity. Since there is overlap in the binding targets of different SH2 domains, the ability to inhibit one SH2 domain specifically will be advantageous for developing target-specific therapeutics. Furthermore, these peptides are the first non-phosphorylated cyclic peptides identified as being able to bind to the SH2 domain of Grb7, and have an advantage over other ligands to SH2 domains in that they do not possess a highly charged phosphate group. It is believed that phosphate groups can lead to poor cell penetration and predicted instability owing to the presence of endogenous phosphatases. As mentioned above, it has recently been suggested that Grb7 SH2 is able to bind to a site (on RndI) that is not phosphorylated (Ref. 30). All other known cellular targets of the Grb7 SH2 bind via a motif containing a pY residue.

Grb7 is an intracellular target; thus, in the further development of these lead Grb7 ligands, it will be important to introduce biologically active peptides inside cells. Although it is possible that Grb7-binding peptides might spontaneously translocate through the cell membrane owing to their small size, additional modifications are likely to be required to enable translocation. Adding a membrane-translocating peptide sequence is a commonly used method for translocation of peptides and even full-length proteins into live cells (Refs 65, 66, 67, 68). There have been several reports describing specific membrane-translocating sequences that effectively deliver peptides to the cytoplasm and the nucleus (Refs 65, 66, 67, 68). Another possible method of delivering peptide inhibitors into a cell is by encapsulation of peptide ligands in liposomes (Refs 69, 70). These peptide-delivery systems are being evaluated to determine which is the most effective at translocating the Grb7-binding peptides across the cell membrane.

Concluding remarks

The improved survival of breast cancer patients with the targeted therapeutic Herceptin, a

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humanised antibody against ErbB2 (Ref. 71), marked a major developmental milestone of targeted therapeutics. A significant reduction in the size of the targeting ligand holds the promise of further improved therapeutic value, as shown by the greater tumour penetration of anti-ErbB2 single-chain (sc)Fv antibodies (25 kDa) compared with that of full-sized (150 kDa) antibodies (Refs 72, 73, 74, 75). At 1–2 kDa, peptide ligands are even smaller, but still retain the selective binding affinity to target molecules and might therefore overcome several of the limitations of antibody therapy. Since antibodies have poor penetration across the cell membrane, peptide inhibitors are ideal for targeting intracellular targets such as Grb7.

Activation of RTKs recruits the SH2 domain of Grb7 and activates an unkown downstream pathway. Overexpression of Grb7 in some cancers appears to initiate invasion and migration of cancer cells. The possibility therefore exists that Grb7-specific inhibitors that bind to the SH2 domain will inhibit the function of Grb7 and, ultimately, the invasion and migration phenotype of a cancer cell. Ongoing research in our laboratory is being conducted to evaluate the anti-migratory effects of the identified Grb7-binding peptides in cancer cells overexpressing Grb7. These Grb7-binding peptides will be further evaluated as lead compounds for drugs that target diseases where Grb7 is overexpressed and might provide useful tools for the elucidation of Grb7 function.

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References

1 Mills, G.B., Lu, Y. and Kohn, E.C. (2001) Linking molecular therapeutics to molecular diagnostics: inhibition of the FRAP/RAFT/TOR component of the PI3K pathway preferentially blocks PTEN mutant cells in vitro and in vivo. Proc Natl Acad Sci U S A 98, 10031-10033, PubMed: 21417775

2 Baselga, J. et al. (1996) Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J Clin Oncol 14, 737-744, PubMed: 96186197

3 Hidalgo, M. et al. (2001) Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. J Clin Oncol 19, 3267-3279, PubMed: 21326212

4 Eckhardt, S.G. et al. (1999) Phase I and pharmacologic study of the tyrosine kinase inhibitor SU101 in patients with advanced solid tumors. J Clin Oncol 17, 1095-1104, PubMed: 20029895

5 Capdeville, R. et al. (2002) Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. Nat Rev Drug Discov 1, 493-502, PubMed: 22116377

6 Vu, C.B. (2000) Recent advances in the design and synthesis of SH2 inhibitors of Src, Grb2 and ZAP-70. Curr Med Chem 7, 1081-1100, PubMed: 20381426

7 Burke, T.R., Jr. et al. (2001) Phosphoryltyrosyl mimetics in the design of peptide-based signal transduction inhibitors. Biopolymers 60, 32-44, PubMed: 21269935

8 Botfield, M.C. and Green, J. (1995)SH2 and SH3 domains: choreographer's of multiple signalling pathways. J Annu Rep Med Chem 227-237

9 Pero, S.C. et al. (2002) Identification of novel non-phosphorylated ligands, which bind selectively to the SH2 domain of Grb7. J Biol Chem 277, 11918-11926, PubMed: 21922846

10 Sadowski, I., Stone, J.C. and Pawson, T. (1986) A noncatalytic domain conserved among cytoplasmic protein-tyrosine kinases modifies the kinase function and transforming activity of Fujinami sarcoma virus P130gag-fps. Mol Cell Biol 6, 4396-4408, PubMed: 87089798

11 Pawson, T. (1988) Non-catalytic domains of cytoplasmic protein-tyrosine kinases: regulatory elements in signal transduction. Oncogene 3, 491-495, PubMed: 90272217

12 Pawson, T., Gish, G.D. and Nash, P. (2001) SH2 domains, interaction modules and cellular wiring. Trends Cell Biol 11, 504-511, PubMed: 21576840

13 Pawson, T. (1994) SH2 and SH3 domains in signal transduction. Adv Cancer Res 64, 87-110,

Accession information: DOI: 10.1017/S1462399403006627; Vol. 5; 10 May 2003

PubMed: 95185362
14 Margolis, B. (1994) The GRB family of SH2 domain proteins. Prog Biophys Mol Biol 62, 223-244, PubMed: 95199592
15 Poy, F. et al. (1999) Crystal structures of the XLP protein SAP reveal a class of SH2 domains with extended, phosphotyrosine-independent sequence recognition. Mol Cell 4, 555-561, PubMed: 20017053
16 Songyang, Z. et al. (1993) SH2 domains recognize specific phosphopeptide sequences. Cell 72, 767-778, PubMed: 93201599
17 Obermeier, A. et al. (1993) Tyrosine 785 is a major determinant of Trk—substrate interaction. Embo J 12, 933-941, PubMed: 93209240
18 Batzer, A.G. et al. (1994) Hierarchy of binding sites for Grb2 and Shc on the epidermal growth factor receptor. Mol Cell Biol 14, 5192-5201, PubMed: 94309639
19 Margolis, B. et al. (1992) High-efficiency expression/cloning of epidermal growth factor-receptor-binding proteins with Src homology 2 domains. Proc Natl Acad Sci U S A 89, 8894-8898, PubMed: 93028373
20 Skolnik, E.Y. et al. (1991) Cloning of PI3 kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases. Cell 65, 83-90, PubMed: 91191565
21 Pawson, T. and Scott, J.D. (1997) Signaling through scaffold, anchoring, and adaptor proteins. Science 278, 2075-2080, PubMed: 98071002
22 Frantz, J.D. et al. (1997) Human GRB-IRbeta/GRB10. Splice variants of an insulin and growth factor receptor-binding protein with PH and SH2 domains. J Biol Chem 272, 2659-2667, PubMed: 97160567
23 Manser, J., Roonprapunt, C. and Margolis, B. (1997) C. elegans cell migration gene mig-10 shares similarities with a family of SH2 domain proteins and acts cell nonautonomously in excretory canal development. Dev Biol 184, 150-164, PubMed: 97287863
24 Ooi, J. et al. (1995) The cloning of Grb10 reveals a new family of SH2 domain proteins. Oncogene 10, 1621-1630, PubMed: 95249278
25 Stein, D. et al. (1994) The SH2 domain protein GRB-7 is co-amplified, overexpressed and in a tight complex with HER2 in breast cancer. Embo J 13, 1331-1340, PubMed: 94185639
26 Manser, J. and Wood, W.B. (1990) Mutations affecting embryonic cell migrations in Caenorhabditis elegans. Dev Genet 11, 49-64, PubMed: 90298583
27 Shen, T.L., Han, D.C. and Guan, J.L. (2002) Association of Grb7 with phosphoinositides and its role in the regulation of cell migration. J Biol Chem 277, 29069-29077, PubMed: 22151071
28 Wojcik, J. et al. (1999) Sequence analysis identifies a ras-associating (RA)-like domain in the N-termini of band 4.1/JEF domains and in the Grb7/10/14 adapter family. Biochem Biophys Res Commun 259, 113-120, PubMed: 99268807
29 He, W. et al. (1998) Grb10 interacts differentially with the insulin receptor, insulin-like growth factor I receptor, and epidermal growth factor receptor via the Grb10 Src homology 2 (SH2) domain and a second novel domain located between the pleckstrin homology and SH2 domains. J Biol Chem 273, 6860-6867, PubMed: 98175951
30 Vayssiere, B. et al. (2000) Interaction of the Grb7 adapter protein with Rnd1, a new member of the Rho family. FEBS Lett 467, 91-96, PubMed: 20130024
31 Janes, P.W. et al. (1997) Structural determinants of the interaction between the erbB2 receptor and the Src homology 2 domain of Grb7. J Biol Chem 272, 8490-8497, PubMed: 97236805
32 Jones, N. et al. (1999) Identification of Tek/Tie2 binding partners. Binding to a multifunctional docking site mediates cell survival and migration. J Biol Chem 274, 30896-30905, PubMed: 99452988
33 Han, D.C., Shen, T.L. and Guan, J.L. (2000) Role of Grb7 targeting to focal contacts and its phosphorylation by focal adhesion kinase in regulation of cell migration. J Biol Chem 275, 28911-28917, PubMed: 20435855
34 Han, D.C. and Guan, J.L. (1999) Association of focal adhesion kinase with Grb7 and its role in cell migration. J Biol Chem 274, 24425-24430, PubMed: 99377086
35 Frisch, S.M. et al. (1996) Control of adhesion-dependent cell survival by focal adhesion kinase. J Cell Biol 134, 793-799, PubMed: 96326682
36 Ilic, D. et al. (1995) Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature 377, 539-544, PubMed: 96013747
37 Zhao, J.H., Reiske, H. and Guan, J.L. (1998) Regulation of the cell cycle by focal adhesion kinase. J Cell Biol 143, 1997-2008, PubMed: 99085075

Accession information: DOI: 10.1017/S1462399403006627; Vol. 5; 10 May 2003

38 Guan, J.L. (1997) Focal adhesion kinase in integrin signaling. Matrix Biol 16, 195-200, PubMed: 98065571
39 Richardson, A. et al. (1997) Inhibition of cell spreading by expression of the C-terminal domain of focal adhesion kinase (FAK) is rescued by coexpression of Src or catalytically inactive FAK: a role for paxillin tyrosine phosphorylation. Mol Cell Biol 17, 6906-6914, PubMed: 98038759
40 Xing, Z. et al. (1994) Direct interaction of v-Src with the focal adhesion kinase mediated by the Src SH2 domain. Mol Biol Cell 5, 413-421, PubMed: 94331781
41 Cobb, B.S. et al. (1994) Stable association of pp60src and pp59fyn with the focal adhesion-associated protein tyrosine kinase, pp125FAK. Mol Cell Biol 14, 147-155, PubMed: 94088511
42 Schaller, M.D. et al. (1994) Autophosphorylation of the focal adhesion kinase, pp125FAK, directs SH2-dependent binding of pp60src. Mol Cell Biol 14, 1680-1688, PubMed: 94158840
43 Chan, P.Y. et al. (1994) A transmembrane-anchored chimeric focal adhesion kinase is constitutively activated and phosphorylated at tyrosine residues identical to pp125FAK. J Biol Chem 269, 20567-20574, PubMed: 94327635
44 Chen, H.C. and Guan, J.L. (1994) Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase. Proc Natl Acad Sci U S A 91, 10148-10152, PubMed: 95024002
45 Chen, H.C. et al. (1996) Phosphorylation of tyrosine 397 in focal adhesion kinase is required for binding phosphatidylinositol 3-kinase. J Biol Chem 271, 26329-26334, PubMed: 96421638
46 Tanaka, S. et al. (1998) A novel variant of human Grb7 is associated with invasive esophageal carcinoma. J Clin Invest 102, 821-827, PubMed: 98376491
47 Tanaka, S. et al. (2000) Grb7 signal transduction protein mediates metastatic progression of esophageal carcinoma. J Cell Physiol 183, 411-415, PubMed: 20259890
48 Kishi, T. et al. (1997) Molecular cloning of human GRB-7 co-amplified with CAB1 and c-ERBB-2 in primary gastric cancer. Biochem Biophys Res Commun 232, 5-9, PubMed: 97236270
49 Tsuda, H. and Hirohashi, S. (1998) Multiple developmental pathways of highly aggressive breast cancers disclosed by comparison of histological grades and c-erbB-2 expression patterns in both the non-invasive and invasive portions. Pathol Int 48, 518-525, PubMed: 98364744
50 Slamon, D.J. et al. (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235, 177-182, PubMed: 87094192
51 Seshadri, R. et al. (1993) Clinical significance of HER-2/neu oncogene amplification in primary breast cancer. The South Australian Breast Cancer Study Group. J Clin Oncol 11, 1936-1942, PubMed: 94015213
52 Gullick, W.J. (1990) The role of the epidermal growth factor receptor and the c-erbB-2 protein in breast cancer. Int J Cancer Suppl 5, 55-61, PubMed: 91078909
53 Oligino, L. et al. (1997) Nonphosphorylated peptide ligands for the Grb2 Src homology 2 domain. J Biol Chem 272, 29046-29052, PubMed: 98030583
54 Hart, C.P. et al. (1999) Potent inhibitory ligands of the GRB2 SH2 domain from recombinant peptide libraries. Cell Signal 11, 453-464, PubMed: 99325905
55 Gay, B. et al. (1999) Selective GRB2 SH2 inhibitors as anti-Ras therapy. Int J Cancer 83, 235-241, PubMed: 99400680
56 Fendly, B.M. et al. (1990) Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product. Cancer Res 50, 1550-1558, PubMed: 90150117
57 Hudziak, R.M. et al. (1989) p185HER2 monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor. Mol Cell Biol 9, 1165-1172, PubMed: 89261720
58 Tanaka, S. et al. (1997) Coexpression of Grb7 with epidermal growth factor receptor or Her2/erbB2 in human advanced esophageal carcinoma. Cancer Res 57, 28-31, PubMed: 97141776
59 Fiddes, R.J. et al. (1998) Analysis of Grb7 recruitment by heregulin-activated erbB receptors reveals a novel target selectivity for erbB3. J Biol Chem 273, 7717-7724, PubMed: 98184886
60 Garcia-Echeverria, C. (2001) Antagonists of the Src homology 2 (SH2) domains of Grb2, Src, Lck and ZAP-70. Curr Med Chem 8, 1589-1604, PubMed: 21446456
61 Atabey, N. et al. (2001) Potent blockade of hepatocyte growth factor-stimulated cell motility, matrix invasion and branching morphogenesis by antagonists of Grb2 Src homology 2 domain interactions. J Biol Chem 276, 14308-14314, PubMed: 21216737

Accession information: DOI: 10.1017/S1462399403006627; Vol. 5; 10 May 2003

62 Garbay, C. et al. (2000) Inhibitors of Ras signal transduction as antitumor agents. Biochem Pharmacol 60, 1165-1169, PubMed: 20464907
63 Furet, P. et al. (1997) Discovery of 3-aminobenzyloxycarbonyl as an N-terminal group conferring high affinity to the minimal phosphopeptide sequence recognized by the Grb2-SH2 domain. J Med Chem 40, 3551-3556, PubMed: 98020393
64 Gay, B. et al. (1999) Effect of potent and selective inhibitors of the Grb2 SH2 domain on cell motility. J Biol Chem 274, 23311-23315, PubMed: 99367461
65 Cussac, D. et al. (1999) A Sos-derived peptidimer blocks the Ras signaling pathway by binding both Grb2 SH3 domains and displays antiproliferative activity. Faseb J 13, 31-38, PubMed: 99091526
66 Kato, D. et al. (1998) Features of replicative senescence induced by direct addition of antennapedia-p16INK4A fusion protein to human diploid fibroblasts. FEBS Lett 427, 203-208, PubMed: 98268328
67 Lin, Y.Z. et al. (1995) 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 270, 14255-14258, PubMed: 95301500
68 Rojas, M., Yao, S. and Lin, Y.Z. (1996) Controlling epidermal growth factor (EGF)-stimulated Ras activation in intact cells by a cell-permeable peptide mimicking phosphorylated EGF receptor. J Biol Chem 271, 27456-27461, PubMed: 97066925
69 Park, J.W. et al. (1997) Immunoliposomes for cancer treatment. Adv Pharmacol 40, 399-435, PubMed: 97360969
70 Park, J.W. et al. (2001) Tumor targeting using anti-her2 immunoliposomes. J Control Release 74, 95-113, PubMed: 21382302
71 Slamon, D. and Pegram, M. (2001) Rationale for trastuzumab (Herceptin) in adjuvant breast cancer trials. Semin Oncol 28, 13-19, PubMed: 21198152
72 Milenic, D.E. et al. (1991) Construction, binding properties, metabolism, and tumor targeting of a single-chain Fv derived from the pancarcinoma monoclonal antibody CC49. Cancer Res 51, 6363-6371, PubMed: 92034771
73 Fujimori, K. et al. (1989) Modeling analysis of the global and microscopic distribution of immunoglobulin G, F(ab')2, and Fab in tumors. Cancer Res 49, 5656-5663, PubMed: 90002920
74 Tahtis, K. et al. (2001) Biodistribution properties of (111)indium-labeled C-functionalized trans-cyclohexyl diethylenetriaminepentaacetic acid humanized 3S193 diabody and F(ab')(2) constructs in a breast carcinoma xenograft model. Clin Cancer Res 7, 1061-1072, PubMed: 21206452
75 Yokota, T. et al. (1992) Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. Cancer Res 52, 3402-3408, PubMed: 92282621
76 Thommes, K. et al. (1999) Identification of Tyr-703 and Tyr-936 as the primary association sites for Grb2 and Grb7 in the c-Kit/stem cell factor receptor. Biochem J 341, 211-216, PubMed: 99306874
77 Keegan, K. and Cooper, J.A. (1996) Use of the two hybrid system to detect the association of the protein-tyrosine-phosphatase, SHPTP2, with another SH2-containing protein, Grb7. Oncogene 12, 1537-1544, PubMed: 96204009
78 Yokote, K. et al. (1996) Grb7 is a downstream signaling component of platelet-derived growth factor alpha- and beta-receptors. J Biol Chem 271, 30942-30949, PubMed: 97094847
79 Pandey, A. et al. (1996) Direct association between the Ret receptor tyrosine kinase and the Src homology 2-containing adapter protein Grb7. J Biol Chem 271, 10607-10610, PubMed: 96209985
80 Kasus-Jacobi, A. et al. (2000) Evidence for an interaction between the insulin receptor and Grb7. A role for two of its binding domains, PIR and SH2. Oncogene 19, 2052-2059, PubMed: 20260602
81 Han, D.C. et al. (2002) EphB1 associates with Grb7 and regulates cell migration. J Biol Chem 277, 45655-45661, PubMed: 22323212
82 Lee, H. et al. (2000) Constitutive and growth factor-regulated phosphorylation of caveolin-1 occurs at the same site (Tyr-14) in vivo: identification of a c-Src/Cav-1/Grb7 signaling cassette. Mol Endocrinol 14, 1750-1775, PubMed: 20525068

Accession information: DOI: 10.1017/S1462399403006627; Vol. 5; 10 May 2003

Further reading, resources and useful contacts

Han, D.C, Shen, T.L and Guan, J.L. (2001) The Grb7 family proteins: structure, interactions with other signaling molecules and potential cellular functions. Oncogene 20, 6315-6321, PubMed: 11607834

Daly, R.J. (1998) The Grb7 family of signalling proteins. Cell Signal 10, 613-618, PubMed: 9794242

Sawyer, T.K. (1998) Src homology-2 domains: structure, mechanisms, and drug discovery. Biopolymers 47, 243-261, PubMed: 9817027

Cody, W.L. et al. (2000) Progress in the development of inhibitors of SH2 domains. Curr Pharm Des 6, 59-98, PubMed: 10637372

A web-based resource created by André Nantel from the Malcolm Whiteway laboratory (Biotechnology Research Institute, National Research Council, Montreal, Canada) gives details on the structure, function and localisation of the Grb7, Grb10 and Grb14 protein family:
http://cbr-rbc.nrc-cnrc.gc.ca/thomaslab/grb7.html

The Tony Pawson lab website at the Samuel Lunenfeld Research institute, Mount Sinai Hospital (Toronto, Ontario, Canada) provides information on SH2, SH3, WW and PH domains, among many others:
http://www.mshri.on.ca/pawson/domains.html

For more information on the structure, function and evolution of SH2 domains, see the website created by Thierry Rose and Gabriel Waksman at the Dept of Biochemistry and Molecular Biophysics, Washington University School of Medicine (MO, USA):
http://biochem.wustl.edu/~sh2-domains/index.html

Features associated with this article

Figure 1. Domain structure of the Grb7 family (fig001spv).
Table 1. Tyrosine kinases bound by the Grb7 SH2 domain (tab001spv).

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