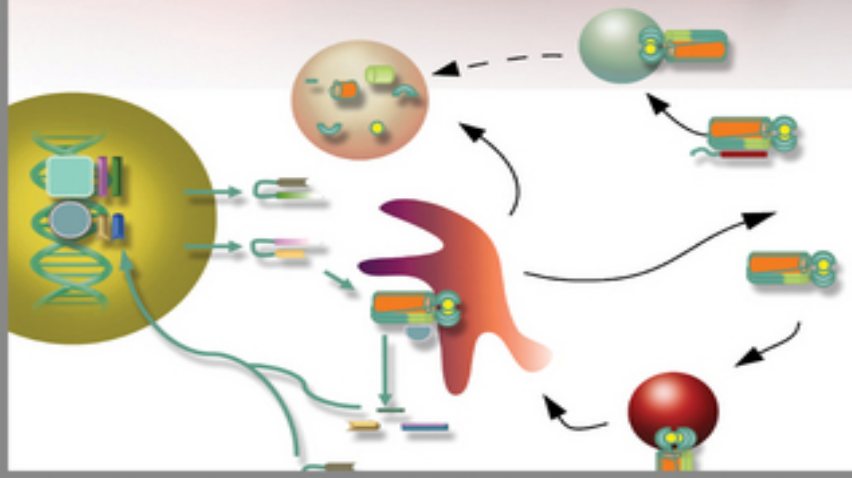
Editor
Amanda Harvey

Cancer Cell Signalling



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Edited by

Amanda Harvey
Biosciences, Brunel University, London, UK

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List of contributors

Andrea R. Daniel
Departments of Medicine (Division of Hematology, Oncology, and Transplantation) and Pharmacology, University of Minnesota, Masonic Cancer Center, 420 Delaware Street, Minneapolis, MN 55455, USA

Christy R. Hagan
Departments of Medicine (Division of Hematology, Oncology, and Transplantation) and Pharmacology, University of Minnesota, Masonic Cancer Center, 420 Delaware Street Minneapolis, MN 55455, USA

Ester M. Hammond
The Cancer Research UK/MRC Gray Institute for Radiation Oncology and Biology, Department of Oncology, The University of Oxford, Oxford, OX3 7DQ, UK

Amanda Harvey
Biosciences, Brunel University, London, Kingston Lane, Uxbridge, UB8 3PH, UK

Christopher Hillyar
The Cancer Research UK/MRC Gray Institute for Radiation Oncology and Biology, Department of Oncology, The University of Oxford, Oxford, OX3 7DQ, UK

Stephen Hiscox
School of Pharmacy and Pharmaceutical Sciences, Cardiff University, CF10 3NB, UK

Emmanouil Karteris
Biosciences, Brunel University, London, Kingston Lane, Uxbridge, UB8 3PH, UK

Todd P. Knutson
Departments of Medicine (Division of Hematology, Oncology, and Transplantation) and Pharmacology, University of Minnesota, Masonic Cancer Center, 420 Delaware Street Minneapolis, MN 55455, USA

Carol A. Lange
Departments of Medicine (Division of Hematology, Oncology, and Transplantation) and Pharmacology, University of Minnesota, Masonic Cancer Center, 420 Delaware Street Minneapolis, MN 55455, USA

Katarzyna Leszczynska
The Cancer Research UK/MRC Gray Institute for Radiation Oncology and Biology, Department of Oncology, The University of Oxford, Oxford, OX3 7DQ, UK

Erald Shehu
Biosciences, Brunel University, London, Kingston Lane, Uxbridge, UB8 3PH, UK

Gudrun Stenbeck
Biosciences, Brunel University, London, Kingston Lane, Uxbridge, UB8 3PH, UK

Maria Thorpe
Biosciences, Brunel University, London, Kingston Lane, Uxbridge, UB8 3PH, UK

David Tree
Biosciences, Brunel University, London, Kingston Lane, Uxbridge, UB8 3PH, UK

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Having read numerous acknowledgments sections in dissertations and theses over the years, it could be assumed that these should be easy to write. However, this is very clearly not the case!

As can be seen from the numerous chapter contributors, this book has not been a solo effort. A sincere thank you goes to all those who have written the drafts, re-written them and produced figures at the editor's whims. Your perseverance and co-operation has been much appreciated. This has been a team effort; we are, of course, supported by our networks of friends and families and we thank them for their patience.

Personally, as the daughter of a biochemistry technician and a Fellow of the Royal Society of Chemistry, a scientific career was hardly unexpected; however, whether it was ‘nature or nurture’, or indeed something entirely different, we are all driven and inspired by a diverse range of individuals. Sadly, not all the people who inspire us are still with us and this book is dedicated to them.

Introduction

One of the on-line encyclopaedias that is frequently used by students defines cell signalling as ‘part of a complex system of communication that governs basic cellular activities and coordinates cell actions’ (Wiki, 2013). As far definitions are concerned, this is a reasonable representation of the role of cell signalling within cells.

Historically, the cell signalling field dates back to the early 1920s when Banting and Best discovered insulin. In the 1950s Italian developmental biologist Rita Levi-Montalcini showed that the chick embryo nervous system was induced to develop by a nerve-growth promoting factor that was released from a transplanted mice tumour. Along with Stanley Cohen the nerve growth promoting factor was subsequently purified, characterised and named nerve growth factor (NGF). Cohen also discovered a second growth factor that promoted opening of the eyelids and tooth eruption. Owing to its action on epithelial cells this factor was termed epidermal growth factor.

This pioneering work paved the way for development of the field we now know as cell signalling, and Stanley Cohen and Rita Levi-Montalcini were jointly awarded Nobel Prizes for their discoveries in 1986 (Nobel Prize Website, 1986). Since then numerous growth factors and their associated receptors have been discovered and their roles in governing the function of normal cells are becoming very well characterised. John Nelson's volume *Structure and Function in Cell Signalling* (Nelson, 2009) provides an excellent foundation for understanding the mechanics of cell signalling. There are some very good definitions and descriptions of the fundamental aspects of ligand–receptor

interactions in addition to, in Chapter 6, an introduction to the concept of signalling cross-talk.

As academics and researchers it is easy to place emphasis on our own specific cell-signalling pathway of interest. It is often the centre of our research specialism and, from a teaching viewpoint, focusing on a single pathway keeps information streamlined and simple and, importantly, from the student perspective, easier to understand.

The reality, however, is far from simple. Many signalling pathways share common effector molecules or feed into common signalling foci or hubs. This allows for altered dynamics within a single pathway to have an effect on a separate, alternate, pathway. Such effects underpin the basis of signalling cross-talk. We now know that stimulation of individual pathways has a far more complex biological outcome than first envisaged.

Much of our knowledge relating to signalling cross-talk has come from cancer biology. In order for a cancer cell to survive, and for a tumour to develop, a number of biological processes need to occur. The cells within the tumour display increased proliferative capabilities and replicative immortality, a reduced capacity for cell death and often a decreased reliance on growth factors. There is also an increase in invasion and metastasis as well as the potential for angiogenesis both in the primary and metastatic tumours. These characteristics or ‘hallmarks’ of cancer cells have been summarised very clearly in Hanahan and Weinberg's reviews of 2000 and 2011 (for those not familiar with cancer biology, these reviews make an excellent starting point for bedtime reading). In both reviews the intracellular circuitry associated with cancer hallmarks has been summarised, although in the decade between the articles the vast increase in knowledge has made it much more difficult to depict the signalling circuits clearly (Hanahan and Weinberg, 2000, 2011).

As our understanding of tumour biology has developed, molecules involved in disrupting normal cell signalling and driving cells towards a more cancerous phenotype have provided the basis for new drug targets, and a number of targeted biological therapies now exist for the treatment of tumours. However, many cancer patients have tumours that are either refractory to treatment or which develop resistance. The amount of research into this area has provided a wealth of information about cross-talk between signalling pathways and compensatory signalling.

This book aims to introduce a number of cell-signalling pathways that are both well characterised and reported to play central roles in the development of a number of different tumour types. Each chapter will focus on an individual pathway, its key components and dysregulation in tumour development. The state of play with respect to current therapies as well as future strategies will also be discussed.

The final chapter of this volume is devoted to signalling cross-talk. Interactions between signalling pathways, compensatory signalling and tumour related issues are discussed with the hope that the reader will not only develop a better appreciation for the role of signalling in disease, but also begin to understand the relevance of cross-talk. The take-home message is intended to be that whilst pathways are important, networks are even more so, especially in the context of cancer development and therapy.

References

Hanahan, D., and Weinberg, R.A. (2000) Hallmarks of cancer. *Cell*, **100** (1), 57–70.

Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. *Cell*, **144** (5), 646–674.

Nelson, J. (2009) *Structure and Function in Cell Signalling*, John Wiley & Sons, Ltd, Chichester.

Nobel Prize Website (1986) http://www.nobelprize.org/nobel_prizes/medicine/laureates/1986/press.html (accessed 10 March 2013).

Wiki (2013) http://en.wikipedia.org/wiki/Cell_signaling (accessed 19 March 2013).

About the companion website

This book is accompanied by a companion website:

www.wiley.com/go/harvey/cancercellsignalling

The website includes:

- Powerpoints of all figures from the book for downloading
- PDFs of tables from the book

Chapter 1

Epidermal growth factor receptor family

Amanda Harvey

Biosciences, Brunel University, London

ErbB receptor ligands bind to their respective receptors, initiating the formation of homo- or heterodimers. The intracellular kinase domain of one receptor trans-phosphorylates the intracellular tyrosine residues on the opposite receptor thereby activating downstream signalling pathways.

At a cellular level, ErbB receptor ligands control a number of processes, including cell cycle progression, proliferation, cell death, protein synthesis, metabolism and differentiation. Physiologically this results in regulation of wound healing, neonatal growth and development as well as the development of adult tissues. Alterations in ErbB receptor signalling can result in oncogenesis in response to increased proliferation and decreased cell death as well as up-regulation of processes required for cell metastasis, such as adhesion, migration, invasion and neo-angiogenesis.

1.1 ErbB receptors and their structure

The epidermal growth factor receptor (EGFR/HER1) and the other family members (c-ErbB2/HER2/neu, ErbB3/HER3 and ErbB4/HER4) are 160–190 kDa transmembrane (type 1) receptor tyrosine kinases. They each comprise extracellular ligand binding and cysteine-rich domains, a transmembrane region, a kinase domain and an intracellular *C*-terminal tail, which contains the multiple tyrosine phosphorylation sites that are required for regulating receptor activation (reviewed in Ferguson, 2008).

EGFR was the first member of the family to be identified; it is a 170 kDa glycoprotein (Carpenter, 1987). Co-purification of the receptor with its growth factor ligand (epidermal growth factor, EGF) was reported in 1979 (McKanna *et al*., 1979), which followed the discovery of EGF in 1972 (Savage *et al*., 1972) and Stanley Cohen's pioneering work showing that EGF bound to the surface of cells (Cohen *et al*., 1975; Carpenter *et al*., 1975, 1978). HER2 was characterised in the 1980s as a 185 kDa protein (Schechter *et al*., 1984) and has been shown to be highly homologous to EGFR (Coussens *et al*., 1985). There are proto-oncogenic and oncogenic forms of HER2 and these differ in sequence by a single amino acid substitution (Bargmann *et al*., 1986). HER2 is the preferred dimerisation partner of the other three family members and it is always available for dimerisation, as it largely exists in normal cells in a monomeric state (Weiner *et al*., 1989a).

ErbB3 and ErbB4 were identified as the third and fourth members of the EGFR/ErbB family in the late 1980s based on their sequence homology with EGFR (Plowman *et al*., 1990, 1993; Kraus *et al*., 1989). Much of the sequence is conserved between the family members, with the highest degree of homology between each receptor and EGFR being in the kinase domain.

Of the family, EGFR and ErbB4 are the only fully functional members. ErbB3 has minimal kinase activity so the

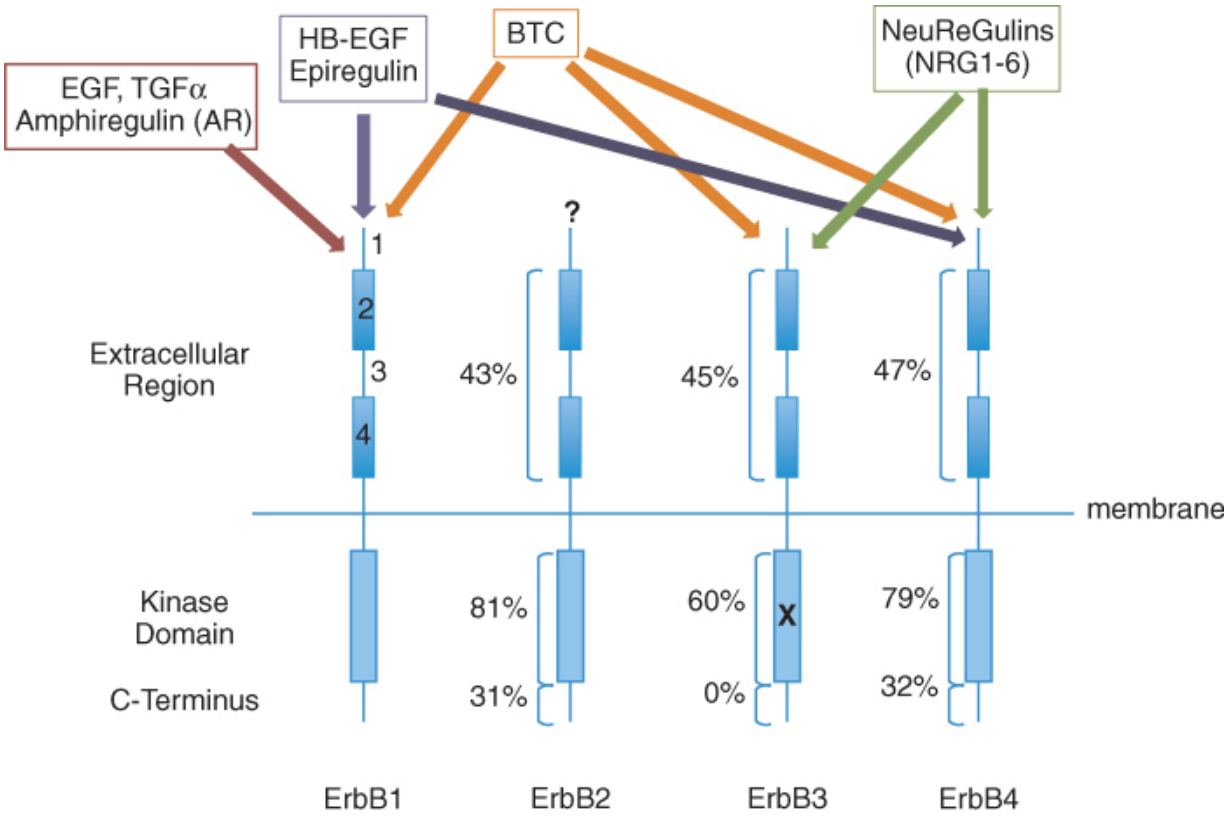
formation of ErbB3 homodimers does not result in active signalling and, as yet, there has been no ligand identified for HER2.

1.2 ErbB ligands

The monomeric growth factor ligands in this peptide family are 45–60 amino acids and they contain six conserved cysteine residues, which are linked by three disulphide bonds. EGF was the first factor to be characterised over 40 years ago (Savage *et al.*, 1972), followed eight years later by transforming growth factor-α (TGFα) (Roberts *et al.*, 1980; Torado *et al.*, 1980). Shortly following its initial discovery, EGF was shown to stimulate DNA synthesis and cell proliferation (Carpenter and Cohen, 1976).

In the 30 years since the discovery of TGFα, the family has grown to over 12 ligands that have different receptor binding preferences and therefore have the ability to regulate different cellular events (Figure 1.1).

Figure 1.1 Schematic representation of the ErbB receptors. All four receptors are depicted and the percentage sequence homology in each domain with the EGFR is indicated. The extracellular region of the receptor has four subdomains, two of which (1 and 3) are involved in ligand binding and two (2 and 4) are cysteine rich and are involved in mediating dimerization. Individual ligands have different binding affinities for specific receptors; note that ErbB2/HER2 does not have a ligand (indicated by ?) and that ErbB3 does not have an active kinase domain (indicated by X), possibly as a result of its reduced homology with EGFR.



EGF, TGFα, epigen and amphiregulin bind to EGFR; epiregulin and heparin binding EGF-like growth factor (HB-EGF) bind to EGFR and HER4; the neuregulins (1–6) have binding preferences for both HER3 and HER4, and betacellulin (BTC) binds to HER2, HER3 and HER4 (reviewed in Eccles, 2011). What is perhaps most striking is that despite appearing to have a functional ligand-binding domain, no ligand has yet been identified that binds HER2 with high affinity, although HER2 will bind with low affinity to the EGF family of ligands.

1.2.1 Ligand production

EGF family ligands are secreted but often require cleavage, unlike ligands for other receptor tyrosine kinases. The ligands are found tethered to the external surface of the cell membrane in pro-forms and require proteolytic cleavage in order to be released. For many ErbB ligands this is carried

out by the disintegrin and metalloproteinase, ADAM17 (Hinkle *et al.*, 2004; Sahin *et al.*, 2004 reviewed in Booth and Smith, 2007) via a process that is known as ectodomain shedding. *In vivo* evidence that ADAM17 acts upstream of EGFR also comes from knock-out mice. Both *ADAM17*$^{-/-}$ and *EGFR*$^{-/-}$ mice display aberrant developmental phenotypes (Wiesen *et al.*, 1999; Jackson *et al.*, 2003; Yamazaki *et al.*, 2003) and EGFR activation only occurred when ADAM17 and amphiregulin were expressed (Sternlicht *et al.*, 2005).

Once soluble, ligands can activate the receptors in paracrine, autocrine or endocrine fashions. This mechanism forms the basis of some types of signalling cross-talk (Chapter 9).

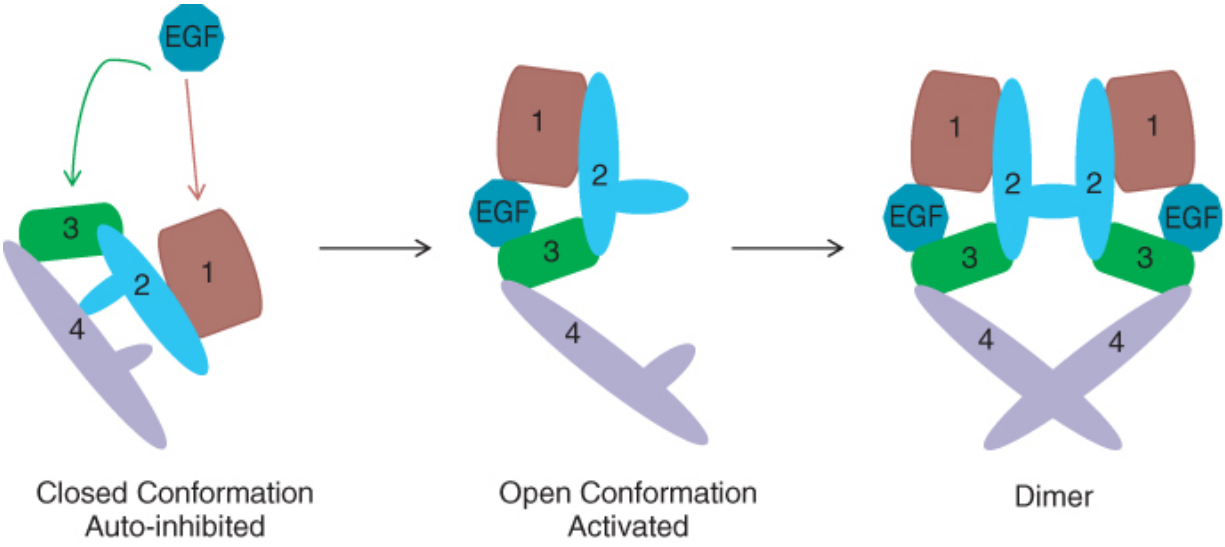
1.2.2 Effects of ligand binding to receptors

The extracellular domains of the receptors are responsible for ligand binding and facilitate most of the dimerisation events. Many of our insights into the mechanisms of ligand binding and the events involved in receptor dimerisation have come from experimental mutations of the receptors (reviewed in Brennan *et al.*, 2000; Ferguson, 2008). Once the ligand has bound to the receptor, an event that occurs with a 1:1 stoichiometry, a change occurs in the conformation of the receptor that facilitates downstream phosphorylation events and signalling transduction.

A number of groups have presented models for investigating ligand–receptor interactions. When the ligand binds to its receptor, a large domain rearrangement occurs that ultimately results in receptor dimerisation. Dimerisation itself is mediated in part, but not solely, by a dimerisation arm or loop that protrudes from the receptor due to the structural rearrangement that takes place upon ligand

binding removing the arm from its intra-molecular tether (Garrett *et al.*, 2002; Ogiso *et al.*, 2002; Ferguson *et al.*, 2003; Greenfield *et al.*, 1989). Exposure of the dimerisation arm initiates the subsequent dimerisation of the receptors with an asymmetric interaction between the intracellular domains (Figure 1.2). In contrast with other signalling pathways (such as IGF, see Chapter 2) ErbB dimerisation involves direct interaction between the receptors, rather than via an association mediated through a divalent ligand that acts as a molecular 'bridge' (reviewed in Ferguson, 2008).

Figure 1.2 Schematic representation of the extracellular domain rearrangement leading to receptor dimerization. In a closed confirmation the receptor is inactive. Ligand (EGF) can bind weakly to subdomain 1, which is not enough to induce receptor activation. However on binding to subdomain 3, the receptor confirmation opens up into an extended confirmation allowing ligand binding to both domains 1 and 3 and exposing the dimerization arm. The extended receptor then dimerizes through interactions that are mediated predominantly through subdomain 2 and, to a lesser extent, subdomain 4 (based on Ferguson *et al.*, 2003).



In addition, ligand binding also brings about additional conformational changes that are required for dimerisation including rotation of part of the receptor (Ogiso *et al.*, 2002 and Ferguson *et al.*, 2003). It is clear that the spatial arrangement of the receptors is important in order that additional contact points can be made at the extracellular interface between the two receptors undergoing dimerisation (Ferguson *et al.*, 2003). The arrangement of these contact points could be central in determining the extent of receptor hetero- or homo-dimerisation.

In 2009 Wilson and colleagues hypothesised that different ErbB ligands would stabilise the extracellular regions of the receptors in slightly different conformations (Wilson *et al.*, 2009). This would affect the spatial arrangement of the contact points at the dimer interface, as well as the position of the dimerisation arm. In addition to influencing the 'choice' of dimerisation partner, subtle changes in spatial stabilisation of the extracellular regions of the receptors could also result in altered interactions between the intracellular domains of the two receptors in the dimer.

Unlike other (non-ErbB-related) receptor tyrosine kinases, the EGFR tyrosine kinase domain does not require activation loop trans-autophosphorylation to promote kinase activation (Gotoh *et al.*, 1992). Instead formation of the asymmetric dimer allosterically activates the dimeric kinase domain (Zhang *et al.*, 2006).

Given this asymmetric event, the accessibility of the cytoplasmic tyrosine residues for trans-phosphorylation by the kinase domain of the opposing dimerisation partner will vary depending on the nature of the interaction and the spatial arrangement of the two intracellular domains. Specificity of tyrosine phosphorylation will determine which downstream intracellular effector/adaptor proteins can bind to the activated receptor, resulting in the activation of different downstream pathways.

There are a total of ten possible receptor combinations resulting in dimer formation, although not all result in active signalling complexes (Table 1.1). ErbB3 homodimers have minimal kinase activity, although each monomer highly augments signalling when in a heterodimer with other members of the family.

Table 1.1 Reported ErbB homo- and hetero-dimers.

	ErbB1	ErbB2	ErbB3	ErbB4
ErbB1	1–1	**1–2**	1–3	
ErbB2		2–2[a]		
ErbB3	3–1	**3–2**	3–3[b]	3–4
ErbB4		**4–2**		4–4

[a]With no known ligand ErbB2 homodimerization only contributes to signalling when ErbB2 is over expressed.
[b]As ErbB3 is kinase inactive, ErbB3 homodimers are not functionally active.

1.3 Downstream signalling molecules and events

Wilson's hypothesis is supported by our biological knowledge. It has been evident for some time that the different receptors are capable of activating different downstream signalling cascades. When ErbB2/ErbB3 heterodimers form, the cytoplasmic tail of ErbB2 activates the Erk-MAPK pathway and ErbB3 activates PI3K-Akt (phosphatidylinositol 3-kinase, PI3K) signalling pathway (Alimandi *et al.*, 1995). These differences come about, in part, by the specificities of each of the tyrosine phosphorylation events on the *C*-terminal tail of the receptor. The variety of adapter molecules that can then potentially bind to, or dock with, each receptor is summarised in Table 1.2. It can be seen from the table that

some signalling effector molecules can dock on all four receptors, potentially at multiple sites, whereas others only have specificity for one receptor or a single site on a limited number of receptors.

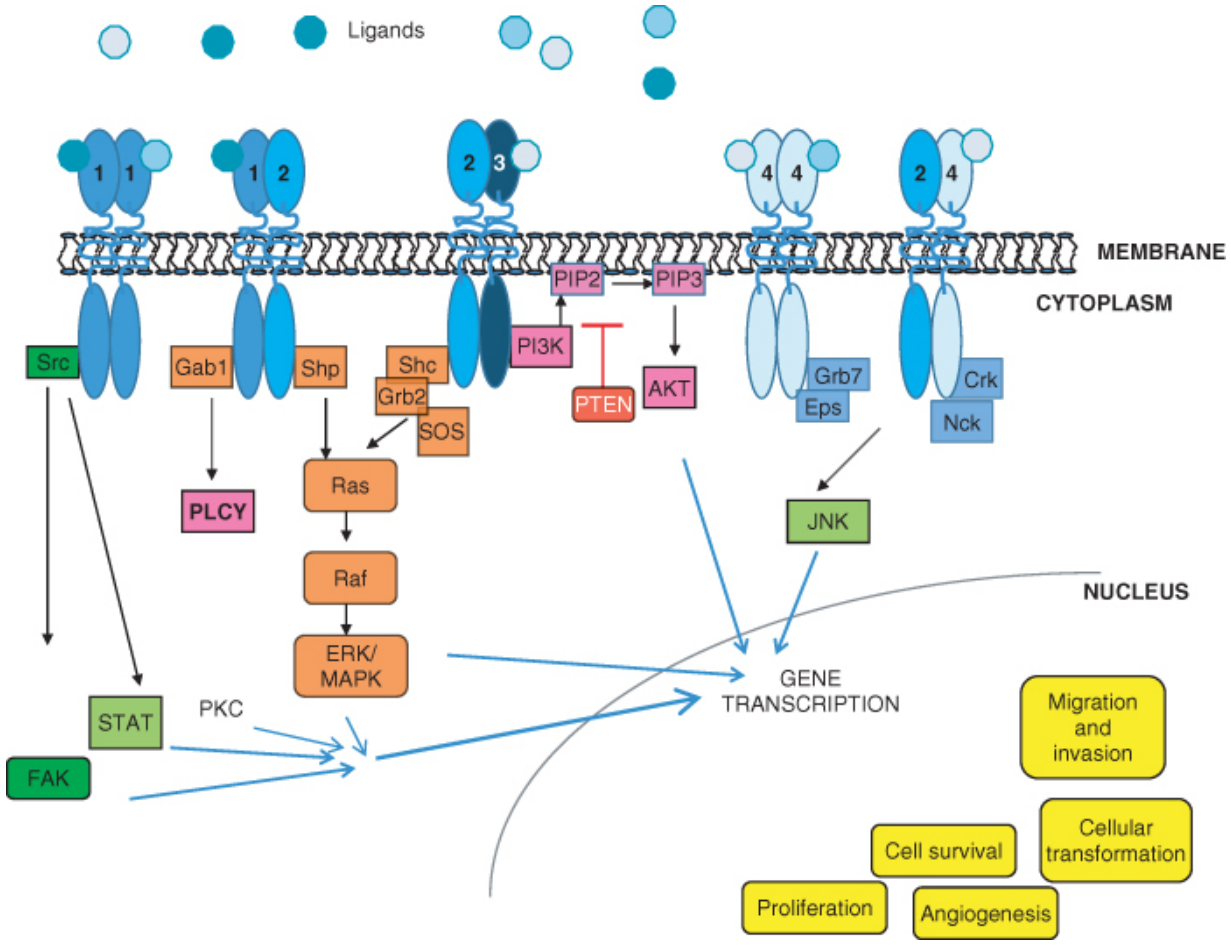
Table 1.2 Potential docking sites for signaling effectors.

Receptor	Signaling effector	Adaptor docking site[a]
EGFR	Shc	Y703 Y974 Y1086 Y1148 Y1173
	STAT5	Y954 Y974
	Crk	Y954 Y974
	PTP-2c	Y974 Y992
	Src	Y974
	PLC gamma	Y992 Y1173
	Cbl	Y1045
	Grb2	Y1068 Y1086 Y1101 Y1148 Y1173
	SHP1	Y1173
HER2	Shc	Y735 Y1005 Y1196 YY1222 Y1248
	SH3BGRL	Y923 Y1196
	PTP-2c	Y1023
	Grb2	Y1139
HER3	p85 PI3-K	Y1054 Y1197 Y1222 Y1276 Y1289
	Grb2	Y1199 Y1262
	Shc	Y1328
HER4	Shc	Y733 Y1188 Y1258 Y1284
	PLC gamma	Y875
	STAT5	Y984
	PTP-2c	Y984
	Crk	Y1022 Y1150
	STAT1	Y1035
	p85 PI3-K	Y1056
	Abl	Y1056 Y1081 Y1150 Y1162 Y1188 Y1242
	Grb2	Y1162 Y1188 Y1202 Y1208 Y1221 Y1242 Y1268
	Cbl	Y1056
	Src	Y1128
	Syk	Y1150 Y1202
	Ras A1	Y1150
	Vav2	Y1162
	Nck	Y1268

[a] There are numerous potential phosphor-tyrosine (Y) docking sites for signalling effectors (adapted from Wilson *et al.*, 2009). Note the number of putative Grb2 docking sites on both EGFR and HER4, and Shc sites on EGFR, HER2 and HER4, although Grb and Shc potentially dock on four receptors. This contrasts with Abl, which docks exclusively on HER4 and p85 PI3-K that is almost exclusive to HER3.

Once adapter molecules have bound to the activated receptors, a number of downstream signalling cascades can be induced (Figure 1.3). EGFR activation by EGF, TGFα, amphiregulin, heregulin and HB-EGF, and ErbB3 activation via neuregulins leads to the activation of phosphatidylinositol 3-kinases (PI3K), resulting in phosphorylation of phosphotatidylinositol 4,5-bisphosphate (PIP_2) to produce phosphatidylinositol 3,4,5-triphosphate (PIP_3). Akt then translocates to the membrane, and the conformational change that is produced when this happens allows Akt to be activated through phosphorylation of its active sites (threonine 308 and serine 473) by phosphoinositide-dependent protein kinase 1 (PDK1) and the mTOR complex 2 (mTORC2) (Sarbassov *et al.*, 2005). Akt activation results in phosphorylation of key downstream targets such as NF-kB, FOXO family members and mTOR complex 1 (mTORC1—Chapter 5) (reviewed in Vivanco and Sawyers, 2002). At a cellular level, apoptosis is inhibited and cell proliferation and growth are induced in response to Akt activation (reviewed in Freudlsperger *et al.*, 2011).

Figure 1.3 Key intracellular binding partners for ErbB receptors. This is a simplistic summary of the key signaling pathways that are initiated on ligand binding and dimerization of ErbB family members. As with subsequent chapters, colour has been used to highlight individual pathways, although it must be noted that many of the adaptor proteins/kinases indicated are frequently shared between different pathways (see Chapters 2, 5, 6, 7 and 9).



Regulation of Akt activation occurs thorough the actions of PTEN (phosphate and tensin homolog deleted on chromosome 10) which antagonises PI3K activity by de-phosphorylating PIP_3 (reviewed in Cully *et al.*, 2006; Carnero *et al.*, 2008).

Docking proteins such as Grb2 and Shc are also capable of binding to phosphorylated residues on the cytoplasmic tail of the receptor (Schulze *et al.*, 2005). This ultimately results in formation of a Grb2/SOS complex initiating removal of GDP from a Ras family member, and activation by substitution with GTP. Ras then activates Raf, which subsequently activates the mitogen-activated-protein-kinase (MAPK) cascade. The outcome of MAPK cascade signaling is increased transcription, as a result of transcriptional activator activation (e.g. myc) (Chuang and

Ng, 1994) and increased translation due to phosphorylation of the 40S ribosomal protein S6 kinase (Shahbazian *et al.*, 2006). Interestingly S6 kinase isoforms are also targets of mTOR activation (Chapter 5).

The Janus kinase (JAK)/signal transducers and activators of transcription (STAT) cascade also regulate cell survival. Members of the JAK/STAT pathway also interact with activated receptors to initiate signalling. As their name suggests, the outcome of signalling results in an increase in transcription, especially of target genes whose protein products are involved in increased proliferation and decreased cell death responses.

1.4 Signalling regulation

1.4.1 Regulation of phosphorylation events

Receptor conformation

In normal cells, the activation of signalling cascades is tightly regulated. The relative levels, as well as the combinations of receptors and growth factors that are available will govern specificity of signalling. The result will be that certain pathways will be activated to a higher or lesser extent than the alternatives based on the nature of receptor dimerisations that occur, and by the conformational changes that result from ligand binding, only allowing specific adaptor proteins to have accessibility to the cytoplasmic tail of the activated receptors (see Section 1.2.2).

Action of phosphatases