Membrane Proteins: The Key Players of a Cancer Cell

Kim R. Kampen

Abstract Membrane proteins are involved in the prognosis of the most common forms of cancer. Membrane proteins are the hallmark of a cancer cell. The overexpressed membrane receptors are becoming increasingly important in cancer cell therapy. Current renewing therapy approaches based on receptor overexpression include; antibody therapy, nanocarrier drug delivery, and fluorescent tumor imaging in surgery. Gene profiling reveals cancer specific signatures and may identify membrane proteins that are related to cancer progression and lead to the development of improved therapy strategies in the future.

Keywords Cancer therapy · Gene expression profiling · Membrane proteins · Nanocarriers · Receptor tyrosine kinases · Surgery

Proteins are the chief regulators of cancer cells and exist intracellular as well as extracellular. Receptor tyrosine kinases (RTKs), integrins, ligands, chemokine transmitters, and cytokine receptors are all kinds of membrane-bound proteins that characterize the cell. This cell signature determines where a cell is capable of. All the membrane-bound proteins are there for a reason; otherwise they would probably be degraded. Cells can regulate important cell functions through these membrane proteins, but they can also be isolated on the basis of this signature. Over time we isolated and characterized more and more different cell types on the basis of the proteins they express on their cell membrane.

K. R. Kampen (✉)
Division of Pediatric Oncology/Hematology,
Department of Pediatrics, Beatrix Children's Hospital,
University Medical Center Groningen, University of Groningen,
P.O. Box 30.001, 9700 RB Groningen, The Netherlands
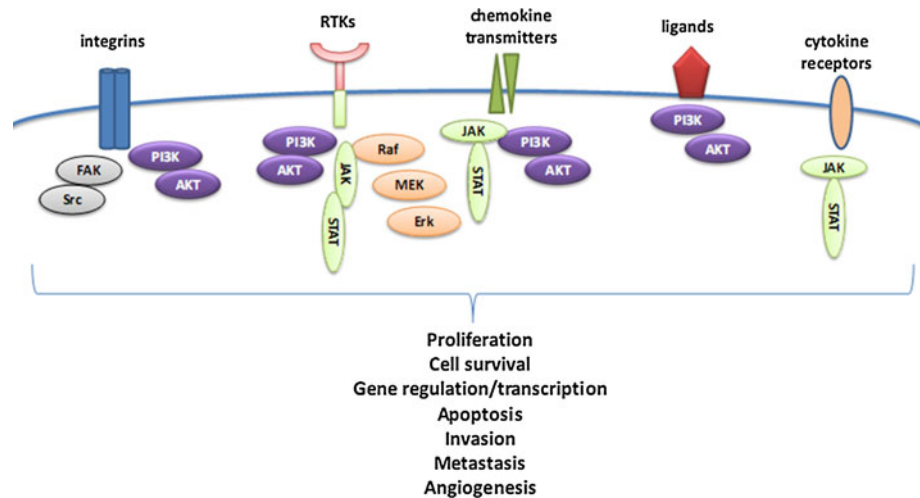e-mail: k.r.kampen@bkk.umcg.nl

Membrane proteins can be activated by secreted factors produced by themselves and by other cells. Membrane proteins can also be activated by cell–cell contact inducing interactions that phosphorylate the membrane protein. These activations might have different consequences for a cell. In most cases, activated membrane proteins will induce intrinsic downstream signaling through phosphorylation of the downstream kinases. The activated signaling pathways demonstrate much overlap in the activating similar kinases as demonstrated in Fig. 1.

Membrane Proteins in Cancer Biology

Kinases—for example, Erk and Akt—are overactive as a result of induced membrane protein phosphorylation and have been found to be important throughout a whole range of cancers (Steelman et al. 2011). If we take a look into the top 5 most frequently occurring forms of cancer, following the World Health Organization, we can observe that increased phosphorylation of Erk and Akt is a common event in all these cancers, as is depicted in Table 1 (Fukuda et al. 2003; Gan et al. 2010; Sah et al. 2004; Serra et al. 2011; Shimizu et al. 2010; Zhang et al. 2011). Of course many translocations result from the production of fusion proteins that contribute to these overactive signaling pathways, but cells need the membrane proteins to the able to transmit the signals and/or to interact with other cells. The membrane receptors involved in Erk/Akt activation in the top 5 most frequent cancers in humans are demonstrated to be dominated by three major receptor signaling pathways. Vascular endothelial growth factor receptors (VEGFRs), epidermal growth factor receptor (EGFR, ErbB-1), and human epidermal growth factor receptor 2 (ErbB-2, i.e. HER2) seem to be the most important receptors; all three are capable of activating

Fig. 1 Cancer cells express many membrane proteins that can all induce downstream signaling through activation of downstream kinases, appointing their overlap. This figure demonstrates how different membrane proteins can induce similar signaling pathways

the same Akt and Erk signaling pathways in cancer. In breast cancer it has been shown that downstream inhibition of the PI3 kinase/Akt pathway induces the phosphorylation of Erk (Serra et al. 2011). This observation has been made for several cancers, and it suggests that targeting a single pathway may not result in the desired effect. Targeting multiple kinases might give a more promising result by inducing apoptosis in an additive way. Another thought is that it might be more effective to inhibit the upstream kinases: the membrane proteins/receptors. As we learn how different receptors in diverse cancers can activate similar downstream signaling pathways, it would be of great value to inhibit multiple receptors instead of a single receptor. Examples of small molecule inhibitors designed for specific targeting of VEGFR-2 (PTK787/ZK222584), VEGFR-2 and platelet-derived growth factor receptor-β (PDGFR-β) (sunitinib), and Raf kinase (sorafenib) have been demonstrated to be "dirty" inhibitors that target multiple membrane receptors (Sosman et al. 2007). Nevertheless, these inhibitors have been proven effective in the treatment of several cancers.

Table 1 Erk/Akt activation in the top 5 most frequent cancers

Cancer	Receptor involvement	Publication
1. Breast cancer	ErbB-2 HER2/neu	Oncogene 2011 (Serra et al. 2011)
2. Prostate cancer	EGFR	Oncogene 2010 (Gan et al. 2010)
3. Lung cancer	EGFR	J Cell Physiol 2011 (Zhang et al. 2011)
4. Colorectum cancer	VEGFRs VEGFR-2	Cancer Research 2003, Chem Biol Interact 2010 (Fukuda et al. 2003; Shimizu et al. 2010)
5. Cervical cancer	EGFR	J Biol Chem 2004 (Sah et al. 2004)

Membrane Proteins Involved in Cancer Prognosis

Membrane-bound proteins have been connected to patient outcome in a variety of cancers. In breast cancer, HER2 expression is associated with poor prognosis, similar to EGFR expression (Noguchi et al. 1994; Toikkanen et al. 1992). Conventional therapy combined with an anti-HER2 antibody treatment has been demonstrated to be effective in HER2-positive breast cancers. This therapy has greatly improved the prognosis of these poor-outcome patients. HER2-negative and metastatic breast cancer tumors are currently being studied in a phase III clinical trial using an anti-VEGFR-2 antibody treatment (ramucirumab, IMC1121b) to sensitize these breast cancer cells (Mackey et al. 2009). In prostate cancer the active form of EGFR is associated with a poor prognosis and resistance to standard treatment (Schlomm et al. 2007). Tumors that express higher amounts of phosphorylated EGFR have been shown to be in a more advanced state of disease, as they are more aggressive tumors. In vitro studies have demonstrated that targeting EGFR shows therapeutic potential, but clinical trials have not yet been started.

Membrane Proteins in Cancer Therapy

Might membrane-bound proteins/receptors be the ideal target to treat cancer? Translocations in cancer cannot be changed; they are a given failure of the system. We can, however, target the cause of the translocation, as we currently do in patients with chronic myeloid leukemia who have the BCR–Abl fusion protein. Unfortunately, targeting the fusion protein cannot cure all chronic myeloid leukemia patients; some of them seem to be resistant to the therapy (Assouline and Lipton 2011). In many cancers we have not yet managed to find the proper therapy to overcome these failure problems. During the last 20 years we have

Table 2 The developed membrane receptor targeted therapy approaches in different cancers

Form of cancer	Therapy	Target	Role/function in cancer
B cell lymphoma, leukemia	Rituximab, Rituxan	CD20	Present on B cells
Colon cancer, head and neck cancer, non–small lung cancer	Cetuximab, Erbitux, Gefitinib	EGFR	Uncontrolled cell proliferation
Breast cancer, gastric cancer	Traztuzumab	HER2/neu, VEGFRs	Present on breast cancer cells, involved in metastasis
Advanced solid tumors, leukemia, metastatic melanoma, hepatocellular carcinoma	PTK787/ZK222584, Vatalanib	PDGFRs, c-Kit, c-FMS	Angiogenesis, lymphangiogenesis
Advanced solid tumors, metastatic breast cancer	Ramucirumab, IMC1121b	VEGFR-2	Angiogenesis

observed an increase in new treatment options that include membrane protein inhibition, as shown in Table 2 (Bonner et al. 2010; Cook et al. 2010; Czuczman and Gregory 2010; Langenberg et al. 2010; Mackey et al. 2009; Metro et al. 2006; Okines et al. 2010; Roboz et al. 2006; Spratlin et al. 2010; Viani et al. 2007; Woyach et al. 2011; Yau et al. 2010; You and Chen 2011). Not all of them are as successful as would be desired. Rituximab is a well-studied and approved therapy for the treatment of B cell lymphomas (Czuczman and Gregory 2010). Rituximab is a human anti-CD20 monoclonal antibody that binds to its target cancer cell on the basis of membrane protein expression of CD20. These examples demonstrate that new treatment options are becoming increasingly specific. New treatment options are developed to increase overall survival rates, decrease recurrence rates, and reduce the risk of side effects. For many diseases we still need improved treatment options; thus the search for membrane-specific signatures remains an important topic. Targeting membrane-bound proteins is a specific way to target a cancer cell. However, a pitfall of the cancer cell phenotype is that the cancer cells arise from "normal" cells and demonstrate kind of the same signature. The gene expression profiles of the leukemic compartment in patients suffering from leukemia demonstrated that genes up-regulated in the leukemic stem cell population are shown to be high in the hematopoietic stem cell compartment from healthy control subjects as well (Gentles et al. 2010). This result demonstrates that the up-regulated genes are most likely involved in self-renewal, quiescence, and other stem cell-specific signatures. In other words, leukemic stem cells seem quite similar to the normal hematopoietic stem cells they arise from.

Nanocarrier Membrane Protein Targeted Therapy in Cancer

A new treatment strategy involving nanocarriers has been tested in a variety of cancers. This method demonstrates an increasing interest in membrane-bound protein overexpression for accurate drug delivery. The receptor-targeted therapy strategy has not been used in clinical trials so far, but it has produced a wide range of therapies already commercially available and studied in vitro and in vivo (Peer et al. 2007). In the case of hepatic cancer, doxorubicin-loaded nanoparticles that specifically target the folate receptor overexpressed on hepatocellular carcinoma cells have been used (Maeng et al. 2010). The folate receptor nanocarrier system has also been proven effective in gliomas, where doxorubicin-loaded polyethylene glycol liposomal nanocarriers were used to target the overexpressed folate receptor (McNeeley et al. 2009). Nanocarrier-targeted therapy in breast and ovarian cancer also introduced a receptor drug delivery system that uses an EGFR-targeting peptide (Milane et al. 2011). This system was shown to be effective in these cancers. The nanocarrier drug delivery system still needs much research before patients might benefit from this therapy approach.

Membrane Protein Fluorescent Imaging for Accurate Tumor Dissection in Surgery

Although cancer cell-specific signatures have been targeted to improve cancer treatment, they are also becoming more and more prominent for fluorescent imaging during surgeries. Breast cancer, ovarian cancer, prostate cancer, gliomas, and so on are cancers that have been monitored by fluorescent dyes to accomplish more precise tumor dissection (Chiun-Wei et al. 2011; Crane et al. 2011; Kantelhardt et al. 2010; Lee et al. 2011; Poptani 2010; Sampath et al. 2007; Smith-Jones et al. 2008; Themelis et al. 2011). In some cases they can use the receptors that are overexpressed on the tumor cell membrane to visualize the exact boundaries of tumors by fluorescent probes or anti-receptor antibodies to enhance the accuracy of dissecting the total tumor. Traztuzumab has demonstrated encouraging results in breast cancer xenograft near-infrared fluorescence imaging, but also showed some aspecific staining that makes tumor

excision inaccurate (Sampath et al. 2007) (Table 2). In ovarian cancer much research has been performed over the last 10 years concerning fluorescence imaging, with innovative results (Crane et al. 2011). For intraoperative fluorescent imaging in ovarian cancer, the folate receptor overexpression on ovarian cancer cells may be used (Smith-Jones et al. 2008). Targeting folate receptor with a fluorescent probe has been demonstrated to clearly distinguish ovarian tumor tissue from normal tissue. Clinical trials are currently in process to evaluate whether this method could be included as a new standard surgical tool for tumor dissection.

Gene Expression Profiling: There is More to Discover

Gene expression profiling of cancers is an important topic in cancer research to evaluate cancer signature profiling and thereby predict patient outcomes. Gene expression profiles demonstrate the significance of membrane protein involvement. Gene expression profiling of tumorigenic breast cancer cells from patients with early breast cancer ($n = 295$) were compared with normal breast epithelium cells (Liu et al. 2007). The invasiveness gene signature (IGS) included 183 genes and was demonstrated to be associated with prognosis in breast cancer ($n = 295$), medulloblastoma ($n = 60$), lung cancer ($n = 62$), and prostate cancer ($n = 21$). Tumors with a correlation not similar to IGS (correlation < 0) were demonstrated to have a better outcome than tumors with a gene expression pattern similar to IGS (correlation > 0). Membrane-bound proteins included in the IGS are XPR1, CD59, LRP2, HSPC163, C5orf18, CDw92, TMC4, ZDHHC2, TICAM2, KDELR3, and ErbB-4. Most of these proteins are still relatively unknown, and the role and function of these membrane proteins remains to be investigated. CD59 has been shown to be involved in colorectal cancer, breast cancer, and prostate cancer (Koretz et al. 1993; Madjd et al. 2003; Xu et al. 2005). In all these cancers CD59 correlates with good patient prognosis. The fact that this gene expression profiling study included early breast cancer patients confirms that CD59 is correlated to good prognosis; however, CD59 is included in the "invasiveness" gene signature. Membrane protein ZDHHC2 is related to cancers as well. ZDHHC2 is found to be mutated in colorectal cancer and hepatocellular carcinomas (Oyama et al. 2000). Therefore, ZDHHC2 research might provide substantial innovative information concerning breast cancer pathogenesis. ErbB-4 is a family member of EGFR (ErbB-1). Alterations in ErbB family members 1 and 2 have been associated to breast cancer tumorigenesis, but less attention has been paid to the biological significance of ErbB-4 signaling in these tumors. The *ErbB-4* gene seems to be hypermethylated in a subgroup of breast cancer patients

(Das et al. 2010). This means that the *ErbB-4* gene is not expressed in a subset of breast cancer tumors, which leads to preventing the tumor cells from going into apoptosis. Results suggest a significant role for *ErbB-4* in breast cancer as a tumor suppressor gene. Analyzing gene expression profiling demonstrates that investigating the function of prognostic important membrane proteins might obtain revealing and promising results for future research.

According to the World Health Organization, cancer claims around 7.5 million lives a year, and its incidence is predicted to continue to rise to over 11 million in 2030. The search for new and better treatment options remains necessary to enhance the overall survival rates of cancer patients. Research has brought us a whole range of new treatment options based on the membrane proteins that cancer cells express on their cell membrane, some of them already clinically used and some of them under investigation. Yet many membrane-bound proteins still need to be discovered, identified, investigated, and characterized to develop more accurate therapies to cure cancer patients or to considerably prolong life while improving the patient's quality of life.

References

Assouline S, Lipton JH (2011) Monitoring response and resistance to treatment in chronic myeloid leukemia. Curr Oncol 18:e71–e83

Bonner JA, Harari PM, Giralt J, Cohen RB, Jones CU, Sur RK, Raben D, Baselga J, Spencer SA, Zhu J, Youssoufian H, Rowinsky EK, Ang KK (2010) Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. Lancet Oncol 11:21–28

Chiun-Wei H, Zibo L, Hancheng C, Tony S, Peter SC (2011) Novel alpha(2)beta(1) integrin-targeted peptide probes for prostate cancer imaging. Mol Imaging 10:284–294

Cook N, Basu B, Biswas S, Kareclas P, Mann C, Palmer C, Thomas A, Nicholson S, Morgan B, Lomas D, Sirohi B, Mander AP, Middleton M, Corrie PG (2010) A phase 2 study of vatalanib in metastatic melanoma patients. Eur J Cancer 46:2671–2673

Crane LM, van Oosten M, Pleijhuis RG, Motekallemi A, Dowdy SC, Cliby WA, van der Zee AG, van Dam GM (2011) Intraoperative imaging in ovarian cancer: fact or fiction? Mol Imaging 10:248–257

Czuczman MS, Gregory SA (2010) The future of CD20 monoclonal antibody therapy in B-cell malignancies. Leuk Lymphoma 51:983–994

Das PM, Thor AD, Edgerton SM, Barry SK, Chen DF, Jones FE (2010) Reactivation of epigenetically silenced *HER4/ERBB4* results in apoptosis of breast tumor cells. Oncogene 29:5214–5219

Fukuda R, Kelly B, Semenza GL (2003) Vascular endothelial growth factor gene expression in colon cancer cells exposed to prostaglandin E2 is mediated by hypoxia-inducible factor 1. Cancer Res 63:2330–2334

Gan Y, Shi C, Inge L, Hibner M, Balducci J, Huang Y (2010) Differential roles of ERK and Akt pathways in regulation of EGFR-mediated signaling and motility in prostate cancer cells. Oncogene 29:4947–4958

Gentles AJ, Plevritis SK, Majeti R, Alizadeh AA (2010) Association of a leukemic stem cell gene expression signature with clinical outcomes in acute myeloid leukemia. JAMA 304:2706–2715

Kantelhardt SR, Caarls W, de Vries AH, Hagen GM, Jovin TM, Schulz-Schaeffer W, Rohde V, Giese A, Arndt-Jovin DJ (2010) Specific visualization of glioma cells in living low-grade tumor tissue. PLoS One 5:e11323

Koretz K, Bruderlein S, Henne C, Moller P (1993) Expression of CD59, a complement regulator protein and a second ligand of the CD2 molecule, and CD46 in normal and neoplastic colorectal epithelium. Br J Cancer 68:926–931

Langenberg MH, Witteveen PO, Lankheet NA, Roodhart JM, Rosing H, van den Heuvel IJ, Beijnen JH, Voest EE (2010) Phase 1 study of combination treatment with PTK 787/ZK 222584 and cetuximab for patients with advanced solid tumors: safety, pharmacokinetics, pharmacodynamics analysis. Neoplasia 12: 206–213

Lee H, Akers W, Bhushan K, Bloch S, Sudlow G, Tang R, Achilefu S (2011) Near-infrared pH-activatable fluorescent probes for imaging primary and metastatic breast tumors. Bioconjug Chem 22:777–784

Liu R, Wang X, Chen GY, Dalerba P, Gurney A, Hoey T, Sherlock G, Lewicki J, Shedden K, Clarke MF (2007) The prognostic role of a gene signature from tumorigenic breast-cancer cells. N Engl J Med 356:217–226

Mackey J, Gelmon K, Martin M, McCarthy N, Pinter T, Rupin M, Youssoufian H (2009) TRIO-012: a multicenter, multinational, randomized, double-blind phase III study of IMC-1121B plus docetaxel versus placebo plus docetaxel in previously untreated patients with HER2-negative, unresectable, locally recurrent or metastatic breast cancer. Clin Breast Cancer 9:258–261

Madjd Z, Pinder SE, Paish C, Ellis IO, Carmichael J, Durrant LG (2003) Loss of CD59 expression in breast tumours correlates with poor survival. J Pathol 200:633–639

Maeng JH, Lee DH, Jung KH, Bae YH, Park IS, Jeong S, Jeon YS, Shim CK, Kim W, Kim J, Lee J, Lee YM, Kim JH, Kim WH, Hong SS (2010) Multifunctional doxorubicin loaded superparamagnetic iron oxide nanoparticles for chemotherapy and magnetic resonance imaging in liver cancer. Biomaterials 31:4995–5006

McNeeley KM, Karathanasis E, Annapragada AV, Bellamkonda RV (2009) Masking and triggered unmasking of targeting ligands on nanocarriers to improve drug delivery to brain tumors. Biomaterials 30:3986–3995

Metro G, Finocchiaro G, Toschi L, Bartolini S, Magrini E, Cancellieri A, Trisolini R, Castaldini L, Tallini G, Crino L, Cappuzzo F (2006) Epidermal growth factor receptor (EGFR) targeted therapies in non-small cell lung cancer (NSCLC). Rev Recent Clin Trials 1:1–13

Milane L, Duan Z, Amiji M (2011) Development of EGFR-targeted polymer blend nanocarriers for combination paclitaxel/lonidamine delivery to treat multi-drug resistance in human breast and ovarian tumor cells. Mol Pharm 8:185–203

Noguchi M, Mizukami Y, Kinoshita K, Earashi M, Thomas M, Miyazaki I (1994) The prognostic significance of epidermal growth factor receptor expression in breast cancer. Surg Today 24:889–894

Okines AF, Dewdney A, Chau I, Rao S, Cunningham D (2010) Trastuzumab for gastric cancer treatment. Lancet 376:1736–1737

Oyama T, Miyoshi Y, Koyama K, Nakagawa H, Yamori T, Ito T, Matsuda H, Arakawa H, Nakamura Y (2000) Isolation of a novel gene on 8p21.3-22 whose expression is reduced significantly in human colorectal cancers with liver metastasis. Genes Chromosomes Cancer 29:9–15

Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R (2007) Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2:751–760

Poptani H (2010) EGFR targeted fluorescence imaging in gliomas. Acad Radiol 17:1–2

Roboz GJ, Giles FJ, List AF, Cortes JE, Carlin R, Kowalski M, Bilic S, Masson E, Rosamilia M, Schuster MW, Laurent D, Feldman EJ (2006) Phase 1 study of PTK787/ZK 222584, a small molecule tyrosine kinase receptor inhibitor, for the treatment of acute myeloid leukemia and myelodysplastic syndrome. Leukemia 20:952–957

Sah JF, Balasubramanian S, Eckert RL, Rorke EA (2004) Epigallocatechin-3-gallate inhibits epidermal growth factor receptor signaling pathway. Evidence for direct inhibition of ERK1/2 and AKT kinases. J Biol Chem 279:12755–12762

Sampath L, Kwon S, Ke S, Wang W, Schiff R, Mawad ME, Sevick-Muraca EM (2007) Dual-labeled trastuzumab-based imaging agent for the detection of human epidermal growth factor receptor 2 overexpression in breast cancer. J Nucl Med 48:1501–1510

Schlomm T, Kirstein P, Iwers L, Daniel B, Steuber T, Walz J, Chun FH, Haese A, Kollermann J, Graefen M, Huland H, Sauter G, Simon R, Erbersdobler A (2007) Clinical significance of epidermal growth factor receptor protein overexpression and gene copy number gains in prostate cancer. Clin Cancer Res 13:6579–6584

Serra V, Scaltriti M, Prudkin L, Eichhorn PJ, Ibrahim YH, Chandarlapaty S, Markman B, Rodriguez O, Guzman M, Rodriguez S, Gili M, Russillo M, Parra JL, Singh S, Arribas J, Rosen N, Baselga J (2011) PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. Oncogene 30:2547–2557

Shimizu M, Shirakami Y, Sakai H, Yasuda Y, Kubota M, Adachi S, Tsurumi H, Hara Y, Moriwaki H (2010) (−)-Epigallocatechin gallate inhibits growth and activation of the VEGF/VEGFR axis in human colorectal cancer cells. Chem Biol Interact 185:247–252

Smith-Jones PM, Pandit-Taskar N, Cao W, O'Donoghue J, Philips MD, Carrasquillo J, Konner JA, Old LJ, Larson SM (2008) Preclinical radioimmunotargeting of folate receptor alpha using the monoclonal antibody conjugate DOTA-MORAb-003. Nucl Med Biol 35:343–351

Sosman JA, Puzanov I, Atkins MB (2007) Opportunities and obstacles to combination targeted therapy in renal cell cancer. Clin Cancer Res 13:764s–769s

Spratlin JL, Cohen RB, Eadens M, Gore L, Camidge DR, Diab S, Leong S, O'Bryant C, Chow LQ, Serkova NJ, Meropol NJ, Lewis NL, Chiorean EG, Fox F, Youssoufian H, Rowinsky EK, Eckhardt SG (2010) Phase I pharmacologic and biologic study of ramucirumab (IMC-1121B), a fully human immunoglobulin G1 monoclonal antibody targeting the vascular endothelial growth factor receptor-2. J Clin Oncol 28:780–787

Steelman LS, Chappell WH, Abrams SL, Kempf RC, Long J, Laidler P, Mijatovic S, Maksimovic-Ivanic D, Stivala F, Mazzarino MC, Donia M, Fagone P, Malaponte G, Nicoletti F, Libra M, Milella M, Tafuri A, Bonati A, Bäsecke J, Cocco L, Evangelisti C, Martelli AM, Montalto G, Cervello M, McCubrey JA (2011) Roles of the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways in controlling growth and sensitivity to therapy-implications for cancer and aging. Aging (Albany NY) 3:192–222

Themelis G, Harlaar NJ, Kelder W et al (2011) Enhancing surgical vision by using real-time imaging of alpha(v)beta (3)-integrin targeted near-infrared fluorescent agent. Ann Surg Oncol. doi: 10.1245/s10434-011-1664-9

Toikkanen S, Helin H, Isola J, Joensuu H (1992) Prognostic significance of HER-2 oncoprotein expression in breast cancer: a 30-year follow-up. J Clin Oncol 10:1044–1048

Viani GA, Afonso SL, Stefano EJ, De Fendi LI, Soares FV (2007) Adjuvant trastuzumab in the treatment of her-2-positive early breast cancer: a meta-analysis of published randomized trials. BMC Cancer 7:153

Woyach JA, Ruppert AS, Heerema NA, Peterson BL, Gribben JG, Morrison VA, Rai KR, Larson RA, Byrd JC (2011) Chemoimmunotherapy with fludarabine and rituximab produces extended overall survival and progression-free survival in chronic lymphocytic leukemia: long-term follow-up of CALGB study 9712. J Clin Oncol 29:1349–1355

Xu C, Jung M, Burkhardt M, Stephan C, Schnorr D, Loening S, Jung K, Dietel M, Kristiansen G (2005) Increased CD59 protein expression predicts a PSA relapse in patients after radical prostatectomy. Prostate 62:224–232

Yau T, Chan P, Pang R, Ng K, Fan ST, Poon RT (2010) Phase 1–2 trial of PTK787/ZK222584 combined with intravenous doxorubicin for treatment of patients with advanced hepatocellular carcinoma: implication for antiangiogenic approach to hepatocellular carcinoma. Cancer 116:5022–5029

You B, Chen EX (2011) Anti-EGFR monoclonal antibodies for treatment of colorectal cancers: development of cetuximab and panitumumab. J Clin Pharmacol. doi:10.1177/0091270010395940

Zhang Y, Wang L, Zhang M, Jin M, Wang X (2011) Potential mechanism of interleukin-8 production from lung cancer cells: an involvement of EGF-EGFR-PI3K-Atk-Erk pathway. J Cell Physiol. doi:10.1002/jcp.22722