Drugs of the Future 2010, 35(11): 909-915

CCC: 0377-8282/2010
DOI: 10.1358/dof.2010.35.11.1524350

REVIEW ARTICLE

FUSION MOLECULES FOR THERAPEUTIC TARGETING OF CANCER

J.T. Pento

Department of Pharmaceutical Sciences, College of Pharmacy, University of Oklahoma, Health Sciences Center, Oklahoma City, Oklahoma, USA

CONTENTS

SUMMARY

Currently, the major cancer chemotherapeutic agents are designed to kill all rapidly growing cells, and chemotherapy is therefore often cytotoxic to normal cells with a high turnover rate, such as intestinal epithelial and hematopoietic cells. A major goal in the development of novel cancer therapeutics over the past decade has been the creation of targeted and more selective modes of chemotherapy in order to reduce the high rate of adverse side effects and enhance therapeutic effectiveness. An important approach to enhancing selectivity of cancer therapeutics has been the combination of a highly selective cancer cell-targeting molecule coupled to a cytotoxic or cytostatic anticancer molecule. Typically, an oncolytic fusion would combine a molecule that targets a protein or receptor that is unique to or highly overexpressed by the cancer cells, together with a drug or treatment which is uniquely cytotoxic to the cancer being treated. Thus, the fusion molecules are especially designed for the treatment of specific types of cancer. This article reviews molecular targeting and molecular fusions that are either currently available or under development and appear to be promising approaches to improve therapeutic effectiveness, while reducing the toxicity associated with cancer chemotherapy.

INTRODUCTION

The magic bullet concept was first described by German physician and scientist Paul Ehrlich, who received the Nobel Prize in Physiology or Medicine in 1908 (1). The magic bullet, as described by Ehrlich, is a highly selective and effective therapeutic agent with few if any side effects. Ehrlich initially developed the concept of a magic bullet as it applies to the medical use of antibodies, and later for the use of arsenicals in the treatment of syphilis. Thus, the magic bullet delivers a lethal force with great velocity and accuracy to a specific target.

Of course there are very few magic bullets in our therapeutic arsenal today, but cancer represents a group of diseases in which there is an urgent need for agents with a much higher degree of therapeutic targeting accuracy. Because a very rapid growth rate is a common characteristic of most cancers, the majority of cancer chemotherapeutic drugs used for the primary treatment of cancer are also damaging or lethal to all cells with a high turnover or growth rate. Accordingly, since many normal cells, such as intestinal epithelial and hematopoietic cells, also have a high rate of turnover, cancer chemotherapy is often associated with serious side effects limiting therapeutic usefulness and effectiveness.

Thus, the development of cancer chemotherapeutics that are specifically targeted to cancer cells has been an important emphasis of cancer therapeutic development during the past 10 to 20 years. This approach should lead to the creation of anticancer drugs that are much more selective, more effective therapeutically and have a much lower rate of adverse side effects.

A major approach employed to enhance the selectivity of cancer therapeutics has been the identification of therapeutic targets that are unique and/or highly overexpressed by the cancer cells. Accordingly, the fusion of the targeting molecule to a cytotoxic molecule provides a mechanism to deliver the cytotoxic agent selectively to cancer cells with much less exposure, and thus, less damage to normal noncancerous tissue. Potentially, this approach should require a much smaller dose of cytotoxic therapy, deliver the drug primarily to the target tissue and increase therapeutic effectiveness. These fusion molecules have also been referred to as "hunter–killer" molecules to describe their dual functionality (2). This article reviews the targeting approaches and fusion molecules that are either currently available or under development and thus promising approaches to improve the treatment of cancer.

Correspondence: J. Thomas Pento, PhD, Department of Pharmaceutical Sciences, College of Pharmacy, University of Oklahoma, Health Sciences Center, Oklahoma City, OK 73117, USA. E-mail: tom-pento@ouhsc.edu.

BIOLOGICAL PATHWAYS TARGETED FOR CANCER THERAPY

Cancer is a genetic disease which usually involves a series of genetic mutations and the activation of various oncogenes in the body (3). These genetic alterations result in the loss of normal regulation of tissue growth and turnover, which in turn results in the rapid proliferation of cancer cells and progression to metastasis of the cancer tissue to multiple organs throughout the body. The metastatic dissemination and development of cancer are most often responsible for the suffering and death associated with this disease. Cancer progression and the metastatic cascade is a very complex process; however, following is a summary of the major steps known to be involved in this process.

Since tumors do not produce vascular tissue, when the growing mass of cancer cells is approximately 1-2 mm in diameter, the process of angiogenesis begins (4). At this stage the cancer cells begin to release proteolytic enzymes which break down the connective tissue sheath surrounding the primary tumor mass. This step permits the cancer cells to become much more invasive and mobile as they begin to move away from the tumor mass and into the surrounding normal tissue, local lymphatic vessels, and eventually the systemic bloodstream. The sequence of events involved in cancer progression and metastasis usually involves the overexpression or upregulation of various growth factors, receptors for these growth factors, tumor neovascularization factors, proteolytic enzymes and deregulators of apoptosis (5). Thus, each of these proteins or pathways represents a potential therapeutic target that may be vital and unique to the growth and progression of each specific type of cancer. The following is a summary of the important cancer-related pathways which are the major focus of cancer-targeting research and drug development.

Angiogenesis

The process of angiogenesis is essential for tumor growth and metastatic progression (6-8). Since the tumor or cancer cell mass cannot produce endothelial cells necessary for the formation of lymphatic and blood vessels, when the cancer cell mass becomes large enough that oxygen and nutrients do not diffuse adequately into the tumor tissue (usually when the cell mass is 1-2 mm in diameter), the cancer cells secrete several factors that stimulate the surrounding normal tissue to produce new blood vessels, which grow into the tumor mass to deliver oxygen and nutrients and carry away waste products, thus permitting the tumor mass to rapidly grow much larger (9).

There are a number of biochemicals produced by tumor cells and surrounding stromal cells that induce vascular growth, enhance the formation, migration and stability of endothelial cells and vascular formation referred to as vascular sprouting (10). The biochemicals known to be involved in tumor angiogenesis include vascular endothelial growth factors (VEGF), including VEGF-A to VEGF-E (11, 12), heparin-binding growth factors 1 and 2 (13-15), platelet endothelial cell adhesion molecule (16, 17), thrombospondin-2 (18-20), various integrins (21-24), neuropilin-1 (25-28) and epidermal growth factor (EGF)-like protein 7 (29-31).

Bevacizumab is a humanized monoclonal antibody that selectively binds to VEGF and inhibits tumor angiogenesis. Bevacizumab was approved in the U.S. in 2004 for the treatment of advanced or metastatic colon cancer. Later, it was approved for non-small cell lung and breast cancers, but it is contraindicated in small cell lung cancer (32).

Growth factors

A variety of peptide growth factors (such as EGF and receptor tyrosine-protein kinase erbB-2 [HER2], an EGF-related receptor) (33, 34), insulin-like growth factor (35-37), transforming growth factor (38-40), platelet-derived growth factor (41-44), keratinocyte growth factor (45-49), as well as the specific tyrosine kinase receptors for these growth factors, are often overexpressed during cancer progression. VEGF is involved in cancer neovascularization, as mentioned above (11, 12), and is therefore also a very important cancer-related growth factor. These growth factors are involved in the regulation of many basic cellular functions; thus, the upregulation of one or several of these growth factors or their receptors will enhance the growth and/or diminish the normal regulation of the transformed cancer cells. Mutational activation and/or amplification of growth factor receptor activity and related signal transduction pathways is known to be involved in the metastatic progression of many types of cancer (50, 51). Targeting receptor kinases with selective inhibitors provides an opportunity to achieve more effective therapeutic intervention with less cytotoxicity (50, 52). For example, it is well established that overexpression of the EGF receptor is predictive of aggressive and metastatic growth in various cancers (53, 54). Accordingly, the development of selective kinase inhibitors represents a major component of current oncolytic drug development (50, 52). Currently, there are approximately 10 tyrosine kinase receptor inhibitors approved for cancer therapy, with many other kinase inhibitors under development. In addition, there are several monoclonal antibodies used to inhibit these growth factor receptors, such as cetuximab, which targets the EGF receptor, and trastuzumab, which targets the HER2 receptor (55, 56).

Apoptosis

Normal cells in the body have a finite life span after which the cells die as a result of programmed cell death, which is referred to as apoptosis. This process plays an important role in embryonic development and in the regulation or homeostasis of normal adult tissue and organ function. Apoptosis is a mechanism by which normal tissue eliminates older or damaged cells without disturbing the surrounding healthy tissue. Downregulation or interference with apoptotic pathways is a fundamental characteristic of cancer cells which permits the rapid growth and uncontrolled progression of cancerous tissue (57).

In normal healthy tissue, apoptosis is regulated by means of two major signaling pathways. First is the intrinsic pathway, which is activated by either developmental signals or cellular stress and damage to vital organelles. Cellular tumor antigen p53 is a natural protein that induces apoptosis in response to DNA damage (58, 59). Other intrinsic signals would include pH changes, infection, heat shock and ionizing radiation. As an example, Bcl-2 signaling in cancer cells inhibits apoptosis by inactivating proapoptotic proteins (60). ABT-263 (navitoclax) is an example of a molecule currently under development that targets the Bcl-2 family of antiapoptotic proteins to allow cells to undergo normal apoptosis (61, 62).

The extrinsic pathway is the second triggering mechanism, which is activated by several external proapoptotic receptor agonists such as TNF-related apoptosis-inducing ligand (Apo-2L/TRAIL) (63, 64). Dulanermin is a molecule that activates the extrinsic pathway by activating death receptors 4 and 5, and which appears to selectively produce apoptosis in cancer cells but not in normal healthy tissue (65, 66).

Proteolytic enzymes

Proteolytic enzymes, such as urokinase, aminopeptidases and metalloproteinases, play an important role in tissue remodeling associated with various normal physiological and pathological processes, such as morphogenesis, angiogenesis and tissue repair. However, these enzymes are released by cancer cells and are directly involved in cancer cell migration and metastasis (3). Proteolytic enzymes break down the adhesion proteins and connective tissue surrounding the primary tumor mass and permit the cancer cells to migrate away from the primary tumor and begin to invade the surrounding normal tissue. Marimastat, which acts as a broad-spectrum matrix metalloproteinase inhibitor, was developed as an antineoplastic drug (67); however, this inhibitor performed poorly in clinical trials (68). Because of the important physiological activity of these enzymes, broad-spectrum inhibitors have produced adverse side effects and undesirable clinical results (69). In spite of these early clinical results, the proteolytic enzymes are still considered to be an important oncolytic target.

Antibodies to tumor-specific antigens

Tumor-specific antigens (TSAs) are proteins expressed on the surface of cancer cells, such as α-fetoprotein on germ cell tumors, carcinoembryonic antigen on colon cancers or mucin-1 and epithelial tumor antigen on breast cancers (70-72). These TSAs are excellent targets for the development of antibodies capable of targeting cancer tissue (73). In addition, such antibodies could be utilized for both diagnostic and therapeutic purposes. TSAs that serve as ideal therapeutic targets are proteins that display tumor-specific expression, appear on the surface of the cancer cells and play a critical role in cancer growth and progression (72). In addition, viruses, bacteria and parasites are known to be involved in the initiation of many cancers; thus, these organisms may also serve as useful targets for antibody development (74). The main problem with the use of TSA antibodies is associated with allergic responses to large nonhuman proteins. However, the development of humanized single-chain antibody fragments greatly reduces the occurrence of adverse immune reactions to antibody therapy. Accordingly, these humanized antibody fragments have been shown to be relatively safe and selective as diagnostic and therapeutic tools (75).

FUSION MOLECULES FOR TARGETING CANCER

Following are examples of fusion proteins that have been created to selectively target cancer cells. Most of the fusion molecules described in this article are prototype molecules and under development for selective oncolytic therapy. A summary of these fusion molecules is presented in Table I.

Angiogenesis

Endostatin is an angiogenesis inhibitor that has been shown to selectively target neovascular endothelial cells and suppress tumor growth. Endostatin was fused with cytosine deaminase, which converts the prodrug 5-flucytosine to 5-fluorouracil, an active cytotoxic agent. The endostatin–cytosine deaminase fusion protein was reported to inhibit tumor growth and increase survival in mice with grafted metastatic tumors. In addition, the endostatin–cytosine deaminase fusion protein reduced the growth of endothelial cells and induced apoptosis of the cancer cells (76).

It has been shown that integrins are specifically associated with neovascularization in tumors. Therefore, a fusion molecule containing the Arg-Gly-Asp peptide motif, which has high binding affinity for $\alpha v\beta 3$ integrins, in combination with the Fc fragment of mouse IgG was created in order to target the Fc portion of IgG to the tumor vasculature and produce an antiangiogenic immune response (77). When examined in an in vivo animal model, this fusion molecule inhibited tumor growth and angiogenesis and enhanced animal survival (78). Isoaspartate-glycine-arginine is another integrin-binding motif. Since integrins are expressed in tumor blood vessels, a fusion of this peptide to TNF was created and observed to inhibit tumor growth, both when given alone or in combination with cytotoxic chemotherapy to tumor-bearing mice. It was suggested that peptides containing this peptide motif may be used to target the tumor vasculature for the delivery of oncolytic drugs and/or tumor imaging agents (79).

Apoptosis

It has been reported that breast cancer cells frequently contain a mutated form of p53 and also that anionic phospholipids are unique to tumor blood vessels (80). Various vectors have been examined to reactivate mutated p53 in cancer tissue. For example, the therapeutic combination of a monoclonal antibody that disrupts tumor vasculature by targeting phospholipids on the surface of tumor endothelial cells and p53 reactivation was examined in a nude mouse xenograft model of advanced breast cancer. Combination treatment was extremely effective in reducing tumor xenograft growth or completely eradicating tumors in this cancer model (80). This treatment was also found to produce a significant induction of apoptosis in tumor cells, as well as destruction of tumor blood vessels in advanced breast and gastric cancers (80, 81).

Proteolytic enzymes

The urokinase-type plasminogen activator (urokinase) receptor has been found to be consistently present at the invasive foci of most human cancers (82). Urokinase appears to be the enzyme primarily responsible for the generation of plasmin activity during the process of extracellular matrix degradation, which is critical to the metastatic process (3). Urokinase consists of an A chain and a B chain, with the A chain responsible for receptor binding (83). Residues 12-32 in the A chain are known to be critical for binding to the receptor (84).

Most cancers have an elevated metabolic requirement for the amino acid methionine compared to normal cells and it is known that methionine-deficient cancer cells arrest in the G_2 and G_1 phases of the cell cycle regardless of the availability of other metabolic precur-

Table I. *Summary of fusion molecules for the treatment of cancer.*

Target	Fusion molecule	Mechanism	Indication	Ref.
Angiogenesis	Endostatin + cytosine	Endothelial cytotoxicity	Metastatic tumors	76
	Arg-Gly-Asp + MAb	Antiangiogenic immune response	Various cancers	77, 78
	Isoaspartate-glycine-arginine +TNF	Tumor necrosis	Various cancers	79
Apoptosis	MAb to mutated p53	p53 reactivation	Breast and gastric cancers	80, 81
Proteolytic enzyme receptor	Urokinase–methioninase	Methionine depletion	Breast, lung, prostate, ovarian and pancreatic cancers	86, 87, 89
Growth factor receptors	Antibody to HER2 and VEGF receptors	Inhibition of growth factor signaling	Breast and colon cancers	90
	EGF–HER2-specific oligopeptide + lidamycin	Inhibition of growth factor signaling, cytotoxic	Ovarian cancer	93
Cancer-specific antibodies	α-GalCer + HER2 antibody	Activation of NKT cells	Lung and other cancers	94
Immuno-photosensitizer	KillerRed + HER2 antibody	Irradiation	Various cancers	96, 97
Cancer imaging	Isoaspartate-glycine-arginine + nanoparticle	Tumor vessel imaging	Renal cancers	79
	OctreoScan® + indium-111	Neuroendocrine imaging	Neuroendocrine tumors	99
	Bombesin + methotrexate + ^{99m}Tc	Tumor imaging	Breast and prostate cancers	102

sors, such as homocystine and folates (85). Thus, a fusion protein containing the amino terminal fragment of human urokinase that binds selectively to the urokinase receptor on the surface of invasive cancer cells and the enzyme L-methioninase was created (86). In culture wounding experiments, this fusion molecule produced a concentration-dependent inhibition of both the migration and proliferation of breast cancer cells over a concentration range of 0.01-1 μM (87). In addition, this fusion was found to bind specifically to urokinase receptors on the surface of the cancer cells (88). Furthermore, this fusion protein was shown to inhibit the proliferation of human lung, prostate, ovarian and pancreatic cancer cells (87, 89).

Growth factors

Bostrom and coworkers (90) developed a novel bifunctional antibody that targets two important growth factor-related targets, HER2 and VEGF, both of which are known to be overexpressed and directly involved in the progression and metastasis of a variety of common cancers (91, 92). While HER2 and VEGF have been targeted individually, Bostrom's approach is a unique application of a protein targeted to prevent the signaling of these growth factor-related targets. It has been demonstrated that this combination molecule targets both growth factors, thus inhibiting the growth and progression of breast and colon cancer xenografts (90).

Another example of a bispecific fusion that specifically targets the EGF receptor and HER2 simultaneously was recently reported (93). This fusion molecule, consisting of two receptor-selective oligopeptides in combination with lidamycin, specifically bound to and was internalized by cancer cells. Furthermore, it was observed that this fusion molecule was more potent and selective than the individual monospecific agents in various cancer cell lines. In addition, this fusion was found to inhibit the growth of human ovarian cancer xenografts in vivo. Therefore, the use of bi- or multispecific compounds is a promising approach to reduce systemic toxicity while enhancing the targeting selectivity and effectiveness of these bifunctional inhibitors as anticancer therapeutic agents.

Antibodies to cancer-specific peptides

α-GalCer is a molecule that activates a subset of natural killer T (NKT) immune cells known to have antitumor activity. A fusion molecule containing α-GalCer bound to an HER2 antibody fragment as the targeting agent was tested in tumor-bearing mice. Treatment with this fusion was shown to inhibit the growth and metastasis of the lung cancer in this animal model. The activity of this fusion protein appears to be produced by the targeting and localization of HER2 within the tumor microenvironment and the accumulation of NKT cells associated with an antitumor immune response. It was suggested that this type of targeting may provide a combination innate and adaptive immune response that may be effective for the treatment of many types of cancer (94).

Photodynamic therapy employing a fusion containing a tumor-specific antibody and chemical photosensitizer has been shown to effectively destroy cancer cells (95). An immuno-photosensitizer recombinant fusion protein of a specific anti-HER2 antibody fragment fused to the phototoxic fluorescent protein KillerRed was created. This fusion protein retained high affinity for the cancer antigen, as well as light activation of the photosensitizer. The fusion molecule was shown to efficiently destroy HER2-expressing tumor cells following light irradiation. It was also demonstrated that there is an additive effect when this fusion molecule is used together with cisplatin, a commonly used antitumor agent (96). It has been suggested that targeted protein conjugates that employ antigen–antibody recognition should provide higher selectivity and less cytotoxicity to normal tissue than photosensitizers used alone (97).

TARGETING USED FOR CANCER IMAGING

Another example of fusion molecules used in the diagnosis and treatment of cancer is the development of fusions containing a targeting molecule together with a radionuclide which can be used for the selective imaging or irradiation of cancer tissue (98). An example of this cancer imaging method is the development of isoaspar-

tate-glycine-arginine, a unique integrin-binding molecule linked to fluorescent nanoparticles. Since integrins are uniquely expressed in the tumor vasculature, this molecule targets tumor blood vessels and was observed to bind to integrins in human renal cell carcinoma tissue and to provide fluorescent images of the tumor tissue (79).

OctreoScan® is a combination of pentetreotide, a peptide that targets neuroendocrine tumors, and the radionuclide indium-111. This compound is used as a molecular imaging agent for the scintigraphic localization of primary and metastatic neuroendocrine tumors containing somatostatin receptors (99). OctreoScan® is reported to be highly selective and sensitive for the detection of a variety of neuroendocrine tumors and metastases (100).

Bombesin and bombesin receptors have been shown to play a role in cancers such as prostate, breast, gastric, bronchial and lung carcinomas (101). Therefore, a novel bombesin analogue derived from the universal sequence of bombesin was conjugated to methotrexate, a commonly used antineoplastic agent, and labeled with technetium-99. This fusion was shown to bind and internalize into breast and prostate cancer cells and displayed good uptake into breast cancer xenografts in mice. This study demonstrates the potential of bombesin–cytotoxic conjugates for the selective targeting and treatment of cancer (102).

CONCLUSIONS

In conclusion, the targeting of tumor cells with selective fusion molecules for the treatment of cancer is a rapidly developing field of study in clinical oncology. This approach should have some significant advantages over current cancer chemotherapy. A number of these uniquely engineered combination molecules are currently under development and in clinical trials, which suggests that many of these agents will be available in the near future to improve the effectiveness and safety of cancer chemotherapy.

DISCLOSURES

J.T. Pento is a coinventor of the urokinase–methioninase fusion protein IP which is described in this manuscript. There is a pending U.S. patent application related to this IP as follows: Harrison, R.G., Pento, J.T. "Conjugation for the specific targeting of anticancer agents to cancer cells and production thereof." U.S. Patent application no. 10/870.832, filed June 17, 2005.

REFERENCES

1. Sulek, K. *[Prize in 1908 awarded to P. Ehrlich and E. Metchnikoff for their work in immunology].* Wiad Lek 1967, 20(11): 1117-8.
2. Ellerby, H.M., Bredesen, D.E., Fujimura, S., John, V. *Hunter-killer peptide (HKP) for targeted therapy.* J Med Chem 2008, 51(19): 5887-92.
3. Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P. Molecular Biology of the Cell. Garland Science, 2002.
4. Chiang, A.C., Massague, J. *Molecular basis of metastasis.* N Engl J Med 2008, 359(26): 2814-23.
5. Ramaswamy, S., Ross, K.N., Lander, E.S., Golub, T.R. *A molecular signature of metastasis in primary solid tumors.* Nat Genet 2003, 33(1): 49-54.
6. Folkman, J. *Tumor angiogenesis: Therapeutic implications.* N Engl J Med 1971, 285(21): 1182-6.
7. Folkman, J. *Role of angiogenesis in tumor growth and metastasis.* Semin Oncol 2002, 29(6, Suppl. 16): 15-8.
8. Cavallo, T., Sade, R., Folkman, J., Cotran, R.S. *Tumor angiogenesis. Rapid induction of endothelial mitoses demonstrated by autoradiography.* J Cell Biol 1972, 54(2): 408-20.
9. Folkman, J., Cotran, R. *Relation of vascular proliferation to tumor growth.* Int Rev Exp Pathol 1976, 16: 207-48.
10. Folkman, J. *Angiogenesis.* Annu Rev Med 2006, 57: 1-18.
11. Folkman, J. *Angiogenesis: Initiation and control.* Ann N Y Acad Sci 1982, 401: 212-27.
12. Nissen, L.J., Cao, R., Hedlund, E.M. et al. *Angiogenic factors FGF2 and PDGF-BB synergistically promote murine tumor neovascularization and metastasis.* J Clin Invest 2007, 117(10): 2766-77.
13. Schwertfeger, K.L. *Fibroblast growth factors in development and cancer: Insights from the mammary and prostate glands.* Curr Drug Targets 2009, 10(7): 632-44.
14. Abate-Shen, C., Shen, M.M. *FGF signaling in prostate tumorigenesis—New insights into epithelial-stromal interactions.* Cancer Cell 2007, 12(6): 495-7.
15. Demirkesen, C., Buyukpinarbasili, N., Ramazanoglu, R., Oguz, O., Mandel, N.M., Kaner, G. *The correlation of angiogenesis with metastasis in primary cutaneous melanoma: A comparative analysis of microvessel density, expression of vascular endothelial growth factor and basic fibroblastic growth factor.* Pathology 2006, 38(2): 132-7.
16. Nickoloff, B.J. *PECAM-1 (CD31) is expressed on proliferating endothelial cells, stromal spindle-shaped cells, and dermal dendrocytes in Kaposi's sarcoma.* Arch Dermatol 1993, 129(2): 250-1.
17. Delisser, H.M. *Targeting PECAM-1 for anti-cancer therapy.* Cancer Biol Ther 2007, 6(1): 121-2.
18. John, A.S., Hu, X., Rothman, V.L., Tuszynski, G.P. *Thrombospondin-1 (TSP-1) up-regulates tissue inhibitor of metalloproteinase-1 (TIMP-1) production in human tumor cells: Exploring the functional significance in tumor cell invasion.* Exp Mol Pathol 2009, 87(3): 184-8.
19. Yee, K.O., Connolly, C.M., Duquette, M., Kazerounian, S., Washington, R., Lawler, J. *The effect of thrombospondin-1 on breast cancer metastasis.* Breast Cancer Res Treat 2009, 114(1): 85-96.
20. Bastian, M., Steiner, M., Schuff-Werner, P. *Expression of thrombospondin-1 in prostate-derived cell lines.* Int J Mol Med 2005, 15(1): 49-56.
21. Giancotti, F.G. *Targeting integrin beta4 for cancer and anti-angiogenic therapy.* Trends Pharmacol Sci 2007, 28(10): 506-11.
22. Mitra, S.K., Schlaepfer, D.D. *Integrin-regulated FAK-Src signaling in normal and cancer cells.* Curr Opin Cell Biol 2006, 18(5): 516-23.
23. Koul, D., Shen, R., Bergh, S. et al. *Targeting integrin-linked kinase inhibits Akt signaling pathways and decreases tumor progression of human glioblastoma.* Mol Cancer Ther 2005, 4(11): 1681-8.
24. Jin, H., Varner, J. *Integrins: Roles in cancer development and as treatment targets.* Br J Cancer 2004, 90(3): 561-5.
25. Lu, L., Zhang, L., Xiao, Z., Lu, S., Yang, R., Han, Z.C. *Neuropilin-1 in acute myeloid leukemia: Expression and role in proliferation and migration of leukemia cells.* Leuk Lymphoma 2008, 49(2): 331-8.
26. Ellis, L.M. *The role of neuropilins in cancer.* Mol Cancer Ther 2006, 5(5): 1099-107.
27. Hansel, D.E., Wilentz, R.E., Yeo, C.J., Schulick, R.D., Montgomery, E., Maitra, A. *Expression of neuropilin-1 in high-grade dysplasia, invasive cancer, and metastases of the human gastrointestinal tract.* Am J Surg Pathol 2004, 28(3): 347-56.

28. Akagi, M., Kawaguchi, M., Liu, W. et al. *Induction of neuropilin-1 and vascular endothelial growth factor by epidermal growth factor in human gastric cancer cells.* Br J Cancer 2003, 88(5): 796-802.
29. Saito, Y., Friedman, J.M., Chihara, Y., Egger, G., Chuang, J.C., Liang, G. *Epigenetic therapy upregulates the tumor suppressor microRNA-126 and its host gene EGFL7 in human cancer cells.* Biochem Biophys Res Commun 2009, 379(3): 726-31.
30. Schmidt, M., De Maziere, A., Smyczek, T. et al. *The role of Egfl7 in vascular morphogenesis.* Novartis Found Symp 2007, 283: 18-28.
31. Wu, F., Yang, L.Y., Li, Y.F., Ou, D.P., Chen, D.P., Fan, C. *Novel role for epidermal growth factor-like domain 7 in metastasis of human hepatocellular carcinoma.* Hepatology 2009, 50(6): 1839-50.
32. Cohen, M.H., Gootenberg, J., Keegan, P., Pazdur, R. *FDA drug approval summary: Bevacizumab (Avastin) plus carboplatin and paclitaxel as first-line treatment of advanced/metastatic recurrent nonsquamous non-small cell lung cancer.* Oncologist 2007, 12(6): 713-8.
33. Hipp, S., Walch, A., Schuster, T. et al. *Activation of epidermal growth factor receptor results in snail protein but not mRNA overexpression in endometrial cancer.* J Cell Mol Med 2009, 13(9B): 3858-67.
34. Tijink, B.M., Laeremans, T., Budde, M. et al. *Improved tumor targeting of anti-epidermal growth factor receptor nanobodies through albumin binding: Taking advantage of modular nanobody technology.* Mol Cancer Ther 2008, 7(8): 2288-97.
35. Bahr, C., Groner, B. *The IGF-1 receptor and its contributions to metastatic tumor growth-novel approaches to the inhibition of IGF-1R function.* Growth Factors 2005, 23(1): 1-14.
36. Chen, Y.W., Boyartchuk, V., Lewis, B.C. *Differential roles of insulin-like growth factor receptor- and insulin receptor-mediated signaling in the phenotypes of hepatocellular carcinoma cells.* Neoplasia 2009, 11(9): 835-45.
37. Sachdev, D., Zhang, X., Matise, I., Gaillard-Kelly, M., Yee, D. *The type I insulin-like growth factor receptor regulates cancer metastasis independently of primary tumor growth by promoting invasion and survival.* Oncogene 2009, 29(2): 251-62.
38. Cheng, N., Bhowmick, N.A., Chytil, A. et al. *Loss of TGF-beta type II receptor in fibroblasts promotes mammary carcinoma growth and invasion through upregulation of TGF-alpha-, MSP- and HGF-mediated signaling networks.* Oncogene 2005, 24(32): 5053-68.
39. Buck, M.B., Knabbe, C. *TGF-beta signaling in breast cancer.* Ann N Y Acad Sci 2006, 1089: 119-26.
40. Wang, S.E., Xiang, B., Guix, M. et al. *Transforming growth factor beta engages TACE and ErbB3 to activate phosphatidylinositol-3 kinase/Akt in ErbB2-overexpressing breast cancer and desensitizes cells to trastuzumab.* Mol Cell Biol 2008, 28(18): 5605-20.
41. Wang, Z., Kong, D., Li, Y., Sarkar, F.H. *PDGF-D signaling: A novel target in cancer therapy.* Curr Drug Targets 2009, 10(1): 38-41.
42. Uren, A., Merchant, M.S., Sun, C.J. et al. *Beta-platelet-derived growth factor receptor mediates motility and growth of Ewing's sarcoma cells.* Oncogene 2003, 22(15): 2334-42.
43. Smith, J.S., Wang, X.Y., Qian, J., Hosek, S.M., Scheithauer, B.W., Jenkins, R.B., James, C.D. *Amplification of the platelet-derived growth factor receptor-A (PDGFRA) gene occurs in oligodendrogliomas with grade IV anaplastic features.* J Neuropathol Exp Neurol 2000, 59(6): 495-503.
44. Jechlinger, M., Sommer, A., Moriggl, R. et al. *Autocrine PDGFR signaling promotes mammary cancer metastasis.* J Clin Invest 2006, 116(6): 1561-70.
45. Nguyen, T.N., Zang, X.P., Pento, J.T. *Keratinocyte growth factor stimulates the migration and proliferation of breast cancer cells in a culture wounding model.* Pharmacol Res 2002, 46(2): 179-83.
46. Zang, X.P., Siwak, D.R., Nguyen, T.X., Tari, A.M., Pento, J.T. *KGF-induced motility of breast cancer cells is dependent on Grb2 and Erk1,2.* Clin Exp Metastasis 2004, 21(5): 437-43.
47. Ishikawa, A., Kudo, M., Nakazawa, N., Onda, M., Ishiwata, T., Takeshita, T., Naito, Z. *Expression of keratinocyte growth factor and its receptor in human endometrial cancer in cooperation with steroid hormones.* Int J Oncol 2008, 32(3): 565-74.
48. Yasuhara, T., Okamoto, A., Kitagawa, T. et al. *FGF7-like gene is associated with pericentric inversion of chromosome 9, and FGF7 is involved in the development of ovarian cancer.* Int J Oncol 2005, 26(5): 1209-16.
49. Kurban, G., Ishiwata, T., Kudo, M., Yokoyama, M., Sugisaki, Y., Naito, Z. *Expression of keratinocyte growth factor receptor (KGFR/FGFR2 IIIb) in human uterine cervical cancer.* Oncol Rep 2004, 11(5): 987-91.
50. Janne, P.A., Gray, N., Settleman, J. *Factors underlying sensitivity of cancers to small-molecule kinase inhibitors.* Nat Rev Drug Discov 2009, 8(9): 709-23.
51. Powers, C.J., McLeskey, S.W., Wellstein, A. *Fibroblast growth factors, their receptors and signaling.* Endocr Relat Cancer 2000, 7(3): 165-97.
52. Zhang, J., Yang, P.L., Gray, N.S. *Targeting cancer with small molecule kinase inhibitors.* Nat Rev Cancer 2009, 9(1): 28-39.
53. Klijn, J.G., Berns, P.M., Schmitz, P.I., Foekens, J.A. *The clinical significance of epidermal growth factor receptor (EGF-R) in human breast cancer: A review on 5232 patients.* Endocr Rev 1992, 13(1): 3-17.
54. Sainsbury, J.R., Farndon, J.R., Needham, G.K., Malcolm, A.J., Harris, A.L. *Epidermal-growth-factor receptor status as predictor of early recurrence of and death from breast cancer.* Lancet 1987, 1(8547): 1398-402.
55. Hudis, C.A. *Trastuzumab—Mechanism of action and use in clinical practice.* N Engl J Med 2007, 357(1): 39-51.
56. Santin, A.D., Bellone, S., Roman, J.J., McKenney, J.K., Pecorelli, S. *Trastuzumab treatment in patients with advanced or recurrent endometrial carcinoma overexpressing HER2/neu.* Int J Gynaecol Obstet 2008, 102(2): 128-31.
57. Kerr, J.F., Wyllie, A.H., Currie, A.R. *Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics.* Br J Cancer 1972, 26(4): 239-57.
58. Kiyosaki, K., Nakada, C., Hijiya, N. et al. *Analysis of p53 mutations and the expression of p53 and p21WAF1/CIP1 protein in 15 cases of sebaceous carcinoma of the eyelid.* Invest Ophthalmol Vis Sci 2010, 51(1): 7-11.
59. Morton, J.P., Timpson, P., Karim, S.A. et al. *Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer.* Proc Natl Acad Sci U S A 2010, 107(1): 246-51.
60. Adams, J.M., Cory, S. *The Bcl-2 apoptotic switch in cancer development and therapy.* Oncogene 2007, 26(9): 1324-37.
61. Tse, C., Shoemaker, A.R., Adickes, J. et al. *ABT-263: A potent and orally bioavailable Bcl-2 family inhibitor.* Cancer Res 2008, 68(9): 3421-8.
62. Shoemaker, A.R., Mitten, M.J., Adickes, J. et al. *Activity of the Bcl-2 family inhibitor ABT-263 in a panel of small cell lung cancer xenograft models.* Clin Cancer Res 2008, 14(11): 3268-77.
63. Ashkenazi, A. *Directing cancer cells to self-destruct with pro-apoptotic receptor agonists.* Nat Rev Drug Discov 2008, 7(12): 1001-12.
64. Chuntharapai, A., Dodge, K., Grimmer, K. et al. *Isotype-dependent inhibition of tumor growth in vivo by monoclonal antibodies to death receptor 4.* J Immunol 2001, 166(8): 4891-8.
65. Kischkel, F.C., Lawrence, D.A., Chuntharapai, A., Schow, P., Kim, K.J., Ashkenazi, A. *Apo2L/TRAIL-dependent recruitment of endogenous FADD and caspase-8 to death receptors 4 and 5.* Immunity 2000, 12(6): 611-20.
66. Ashkenazi, A., Holland, P., Eckhardt, S.G. *Ligand-based targeting of apoptosis in cancer: The potential of recombinant human apoptosis ligand*

2/tumor necrosis factor-related apoptosis-inducing ligand (rhApo2L/TRAIL). J Clin Oncol 2008, 26(21): 3621-30.

67. Millar, A.W., Brown, P.D., Moore, J., Galloway, W.A., Cornish, A.G., Lenehan, T.J., Lynch, K.P. *Results of single and repeat dose studies of the oral matrix metalloproteinase inhibitor marimastat in healthy male volunteers.* Br J Clin Pharmacol 1998, 45(1): 21-6.
68. Sparano, J.A., Bernardo, P., Stephenson, P., Gradishar, W.J., Ingle, J.N., Zucker, S., Davidson, N.E. *Randomized phase III trial of marimastat versus placebo in patients with metastatic breast cancer who have responding or stable disease after first-line chemotherapy: Eastern Cooperative Oncology Group trial E2196.* J Clin Oncol 2004, 22(23): 4683-90.
69. Coussens, L.M., Fingleton, B., Matrisian, L.M. *Matrix metalloproteinase inhibitors and cancer: Trials and tribulations.* Science 2002, 295(5564): 2387-92.
70. Niv, Y. *MUC1 and colorectal cancer pathophysiology considerations.* World J Gastroenterol 2008, 14(14): 2139-41.
71. Wreschner, D.H., Hareuveni, M., Tsarfaty, I. et al. *Human epithelial tumor antigen cDNA sequences. Differential splicing may generate multiple protein forms.* Eur J Biochem 1990, 189(3): 463-73.
72. Lucas, S., Coulie, P.G. *About human tumor antigens to be used in immunotherapy.* Semin Immunol 2008, 20(5): 301-7.
73. Siu, D. *Cancer therapy using tumor-associated antigens to reduce side effects.* Clin Exp Med 2009, 9(3): 181-98.
74. Accardi, L., Di Bonito, P. *Antibodies in single-chain format against tumour-associated antigens: Present and future applications.* Curr Med Chem 2010, 17(17): 1730-55.
75. Weiner, L.M., Dhodapkar, M.V., Ferrone, S. *Monoclonal antibodies for cancer immunotherapy.* Lancet 2009, 373(9668): 1033-40.
76. Ou-Yang, F., Lan, K.L., Chen, C.T. et al. *Endostatin-cytosine deaminase fusion protein suppresses tumor growth by targeting neovascular endothelial cells.* Cancer Res 2006, 66(1): 378-84.
77. Li, J., Ding, M., Fu, Q., Tan, H., Xie, X., Zhong, Y. *A novel strategy to graft RGD peptide on biomaterials surfaces for endothelization of small-diamater vascular grafts and tissue engineering blood vessel.* J Mater Sci Mater Med 2008, 19(7): 2595-603.
78. Li, J., Ji, J., Holmes, L.M. et al. *Fusion protein from RGD peptide and Fc fragment of mouse immunoglobulin G inhibits angiogenesis in tumor.* Cancer Gene Ther 2004, 11(5): 363-70.
79. Curnis, F., Sacchi, A., Gasparri, A. et al. *Isoaspartate-glycine-arginine: a new tumor vasculature-targeting motif.* Cancer Res 2008, 68(17): 7073-82.
80. Liang, Y., Besch-Williford, C., Benakanakere, I., Thorpe, P.E., Hyder, S.M. *Targeting mutant p53 protein and the tumor vasculature: An effective combination therapy for advanced breast tumors.* Breast Cancer Res Treat 2010, Epub ahead of print.
81. Zheng, T., Meng, X., Wang, J. et al. *PTEN- and p53-mediated apoptosis and cell cycle arrest by FTY720 in gastric cancer cells and nude mice.* J Cell Biochem 2010, 111(1): 218-28.
82. Ellis, V., Pyke, C., Eriksen, J., Solberg, H., Dano, K. *The urokinase receptor: involvement in cell surface proteolysis and cancer invasion.* Ann N Y Acad Sci 1992, 667: 13-31.
83. Stoppelli, M.P., Corti, A., Soffientini, A., Cassani, G., Blasi, F., Assoian, R.K. *Differentiation-enhanced binding of the amino-terminal fragment of human urokinase plasminogen activator to a specific receptor on U937 monocytes.* Proc Natl Acad Sci U S A 1985, 82(15): 4939-43.
84. Appella, E., Robinson, E.A., Ullrich, S.J., Stoppelli, M.P., Corti, A., Cassani, G., Blasi, F. *The receptor-binding sequence of urokinase. A biological function for the growth-factor module of proteases.* J Biol Chem 1987, 262(10): 4437-40.
85. Kokkinakis, D.M., von Wronski, M.A., Vuong, T.H., Brent, T.P., Schold, S.C., Jr. *Regulation of O6-methylguanine-DNA methyltransferase by methionine in human tumour cells.* Br J Cancer 1997, 75(6): 779-88.
86. Peron, K., Jones, T.N., Gauthier, S.A. et al. *Targeting of a novel fusion protein containing methioninase to the urokinase receptor to inhibit breast cancer cell migration and proliferation.* Cancer Chemother Pharmacol 2003, 52(4): 270-6.
87. Palwai, N.R., Zang, X.P., Harrison, R.G., Pento, J.T. *Influence of L-methioninase targeted to the urokinase receptor on the proliferation and motility of lung and prostate cancer cells.* Anticancer Res 2007, 27(5A): 3435-9.
88. Zang, X.P., Palwai, N.R., Lerner, M.R., Brackett, D.J., Pento, J.T., Harrison, R.G. *Targeting a methioninase-containing fusion protein to breast cancer urokinase receptors inhibits growth and migration.* Anticancer Res 2006, 26(3A): 1745-51.
89. Palwai, N.R., Zang, X.P., Harrison, R.G., Benbrook, D., Pento, J.T. *Selective growth inhibition of cancer cells by L-methioninase-containing fusion protein targeted to the urokinase receptor.* Pharmacology 2009, 84(5): 271-5.
90. Bostrom, J., Yu, S.F., Kan, D. et al. *Variants of the antibody herceptin that interact with HER2 and VEGF at the antigen binding site.* Science 2009, 323(5921): 1610-4.
91. Moasser, M.M., Basso, A., Averbuch, S.D., Rosen, N. *The tyrosine kinase inhibitor ZD1839 ("Iressa") inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells.* Cancer Res 2001, 61(19): 7184-8.
92. Folkman, J. *Tumor suppression by p53 is mediated in part by the antiangiogenic activity of endostatin and tumstatin.* Sci STKE 2006, 2006(354): pe35.
93. Guo, X.F., Zhu, X.F., Shang, Y., Zhang, S.H., Zhen, Y.S. *A bispecific enediyne-energized fusion protein containing ligand-based and antibody-based oligopeptides against epidermal growth factor receptor and human epidermal growth factor receptor 2 shows potent antitumor activity.* Clin Cancer Res 2010, 16(7): 2085-94.
94. Stirnemann, K., Romero, J.F., Baldi, L. et al. *Sustained activation and tumor targeting of NKT cells using a CD1d-anti-HER2-scFv fusion protein induce antitumor effects in mice.* J Clin Invest 2008, 118(3): 994-1005.
95. Verma, S., Watt, G.M., Mai, Z., Hasan, T. *Strategies for enhanced photodynamic therapy effects.* Photochem Photobiol 2007, 83(5): 996-1005.
96. Serebrovskaya, E.O., Edelweiss, E.F., Stremovskiy, O.A., Lukyanov, K.A., Chudakov, D.M., Deyev, S.M. *Targeting cancer cells by using an antireceptor antibody-photosensitizer fusion protein.* Proc Natl Acad Sci U S A 2009, 106(23): 9221-5.
97. van Dongen, G.A., Visser, G.W., Vrouenraets, M.B. *Photosensitizer-antibody conjugates for detection and therapy of cancer.* Adv Drug Deliv Rev 2004, 56(1): 31-52.
98. Okarvi, S.M. *Peptide-based radiopharmaceuticals and cytotoxic conjugates: potential tools against cancer.* Cancer Treat Rev 2008, 34(1): 13-26.
99. Rufini, M., Calcagni, M., R., B. *Imaging of neuroendocrine tumors.* Sem Nucl Med 2008, 36: 228-47.
100. Lebtahi, R., Cadiot, G., Sarda, L. et al. *Clinical impact of somatostatin receptor scintigraphy in the management of patients with neuroendocrine gastroenteropancreatic tumors.* J Nucl Med 1997, 38(6): 853-8.
101. Reubi, J.C., Wenger, S., Schmuckli-Maurer, J., Schaer, J.C., Gugger, M. *Bombesin receptor subtypes in human cancers: Detection with the universal radioligand (125)I-[D-TYR(6), beta-ALA(11), PHE(13), NLE(14)] bombesin(6-14).* Clin Cancer Res 2002, 8(4): 1139-46.
102. Okarvi, S.M., Al Jammaz, I. *Synthesis and evaluation of a technetium-99m labeled cytotoxic bombesin peptide conjugate for targeting bombesin receptor-expressing tumors.* Nucl Med Biol 2010, 37(3): 277-88.