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Angiogenesis and P-glycoprotein: Their Roles in Cancer

Alain Thibodeau, Ph.D.

ATRIUM Biotechnologies Inc., Quebec, Canada

Cancer is a major cause of death worldwide. Although nowadays most cancer prognosis tend to be more and more optimistic due to earlier diagnosis, improved knowledge and technological advances, the overall incidence rate of cancer is still progressing. At the cellular level, tumors have strategies to sustain their growth and escape treatment attempts. Through the coordinated action of growth factors and proteolytic enzymes, cancer cells command the expansion of the vascular bed in order to ensure delivery of those nutriments essential to their growth. The process is known as angiogenesis and relies on the production of the vascular endothelial growth factor (VEGF) and activation of some matrix metalloproteinases (MMP). Angiogenesis once established provides escaping ways through which some cancer cells can migrate to form new colonies at distant sites. Chemotherapy is most commonly used to fight cancer. Chemotherapeutic agents (CA) are aimed at killing the rapidly growing cancer cells by interfering with major intracellular mechanisms essential for cell division. Unfortunately, CA have limited potential for cancer cure due to the development of chemotherapeutic drug resistance. Some cancer cells express pumps (P-gp) on their surface which can actively extrude the CA and so allow cells to escape death. In order to win the battle over cancer we should thus use multiple strategies. Starving cells by preventing angiogenesis is a promising avenue and is the focus of intense clinical research. Preventing the development of drug resistance by diverting P-gp pumps activity is another inventive alternative. The Liquid Cartilage Extract (LCE) and the Chemotherapy Active Support Technology (CAST) are all-natural diet supplementations carefully formulated to provide extensive support to standard therapy. The LCE inhibits activation of MMP activity and opposes activation of signalling events trigger by VEGF. These two actions of LCE are most probably at the basis of the growth inhibition observed *in vitro* when endothelial cells are cultured in its presence. The CAST product is a potent inhibitor of P-gp pump activity in cell culture assays and inactivating P-gp pumps in the presence of CAST was shown to optimize CA accumulation in cancer cells. Moreover, CAST also showed inhibitory activity towards MMP. By blocking VEGF signalling and MMP activity, LCE and CAST shall prevent the development of angiogenesis normally driven by cancer cells. This would minimize the tumor size and prevent formation of metastasis. By paralysing P-gp pumps action, CAST shall additionally maintain the cancer cells sensitivity to CA. Taken together, LCE and CAST have complementary actions that may support and optimize any standard therapy.

Corresponding author : Alain thibodeau, ATRIUM Biotechnologies Inc., Quebec, Canada

INTRODUCTION

Cancer is a pandemic disorder for which the incidence rate constantly increases. Several factors such as nutrition, exposure to environmental stresses and genetic background are known to influence the rate of cancer and the pattern of organs afflicted. Cancer negatively impacts the quality of life not only of patients but also of close relatives. However, thanks to modern medical advancements, the mortality rate of several cancers has diminished. This encouraging trend mostly relies on earlier clinical diagnosis and improved therapies. Moreover, the improvement of society's education level succeeded to arise population awareness over healthier practices of life including balanced nutrition.

At the cellular level, there are startegies by which a tumor can encourage its growth or escape treatment attempts. Angiogenesis, the formation of new blood vessels, is induced by tumors and serves to deviates vital nutriments and oxygen to the tumor itself to support its growth. Blood vessel proximity also represents a pathway through which tumor cells can escape and disseminate to distant organs and colonize as metastases. While feeding on nutrient substances conveyed by blood vessels, the tumor is also able to activate special mechanisms to eliminate molecules that represent a threat for its own survival. P-glycoprotein (P-gp) pumps are part of this defense weapon that cancers used to extrude chemotherapeutic agents (CA) out of tumor cells. P-gp action eventually lead to CA resistance and thus seriously hampers with standard chemotherapy.

This paper will discuss the roles of angiogenesis and P-gp in tumor growth. It will also describes recent advances in nutrition technology that show promises in modulating angiogenesis and P-gp activities.

Angiogenesis and Tumor Growth

Angiogenesis is the formation of new blood vessels from preexisting ones. It is a complex process that involves several molecular and cellular mechanisms (Fig. 1).

Various triggering events (signal source) can induce the process of angiogenesis. Probably the most characterized pro-angiogenic factor is the Vascular Endothelial Growth factor (VEGF). VEGF is produced and secreted by tumor cells and reaches neighboring established blood vessels (step I). VEGF is a factor that stimulates endothelial cell proliferation and migration and thus represent a key factor in the promotion of tumor vascularization. Proliferation and migration of endothelial cells is the first cellular event observed during the angiogenesis process.

During the initial phase of their activation process, endothelial cells will secrete specialized enzymes known as matrix metalloproteinases (MMP). These MMPs are required to digest the collagen fibers present in the basement membrane. This

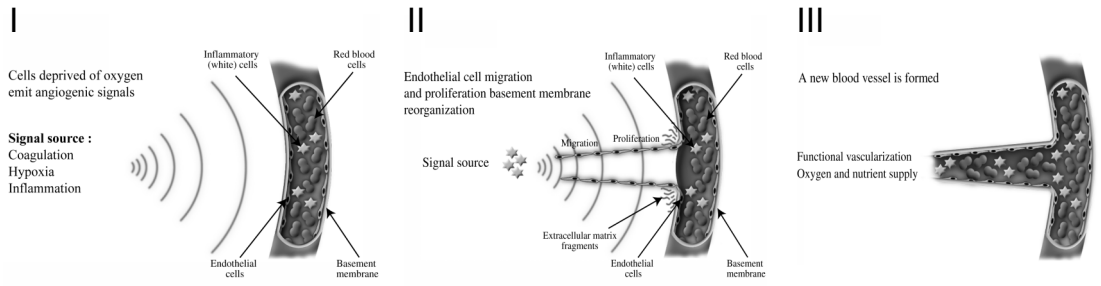


Fig. 1. Angiogenesis: A complex process.

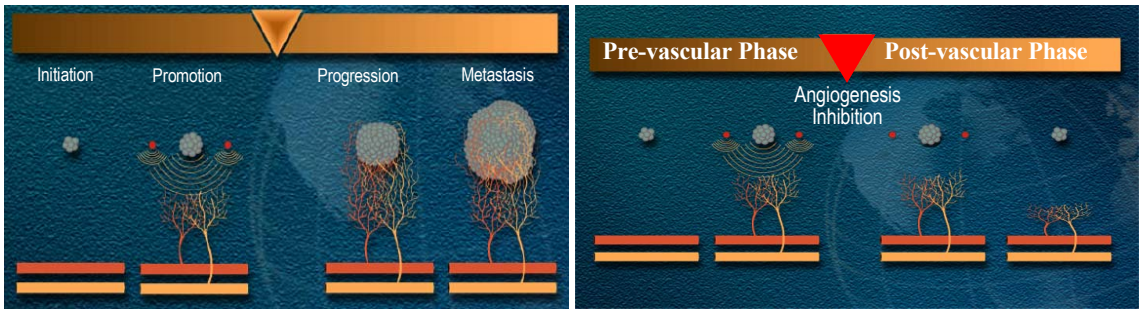


Fig. 2. Angiogenesis and tumor growth.

creates a breach through which proliferating endothelial cells can migrate in the direction of the signal source (step II). Along their path, endothelial cells produce an extracellular matrix that is assembled into an organized basement membrane, leading to the formation of a functional new blood vessel (step III). If the signal comes from tumor cells, the angiogenesis process may result in the vascularization of the tumor, thereby promoting tumor growth (Fig. 2).

Formation of new blood vessels brings oxygen and nutrients to the microscopic tumor cell mass. This leads to tumor cell proliferation and primary tumor growth clinically palpable sizes (Fig. 2). Angiogenesis also allows for tumor dissemination. Individual tumor cells may break out form the parent tumor, enter the circulation and migrate away to colonize distant target organs as metastasis. A daughter tumor may develop through a second cycle of blood vessel recruitment. This scenario can be reiterated until the host can no longer survive this form of parasitism (Zetter 1998).

Angiogenesis inhibition opens doors to new possibilities to interfere with tumor growth. Indeed, blocking the progression of new blood vessels recruitment in the direction of a tumor, attracted by VEGF signals, will shut down tumor nourishment. In this way, suffocating tumor cells will progressively enter a phase of necrosis and with the help of the immune system be annihilated.

LCE Biological Actions

LCE is an aqueous extract that contains natural inhibitors of angiogenesis. LCE prevents the angiogenesis process by inhibiting the enzymatic action of Matrix Metalloproteinases (MMPs) and by interfering with the activation of the pro-angiogenic factor VEGF. LCE is obtained through sophisticated ultrafiltration steps that are part of a Atrium's patented manufacturing process. The oral bioavailability of LCE was previously demonstrated in a human clinical trial (Berbari et al, 1999).

Inhibition of Endothelial Cell Proliferation

Since proliferation of endothelial cells is a key event in the course of angiogenesis, the anti-angiogenic potential of LCE was investigated by looking at its effect on human endothelial cell (HUVEC) proliferation *in vitro*. Results were expressed in % of growth inhibition as compared to cells grown in absence of LCE. As shown here (Fig. 3), LCE dose-dependently inhibits human umbilical vein endothelial cells (HUVEC) growth. Inhibition was almost completed at a concentration of 10 mg/ml.

Inhibition of MMP-2 Activity by LCE

MMP activity also being important for angiogenesis, we seek for a potentiel effect of LCE on the enzymatic activity of MMP-2, a representative of

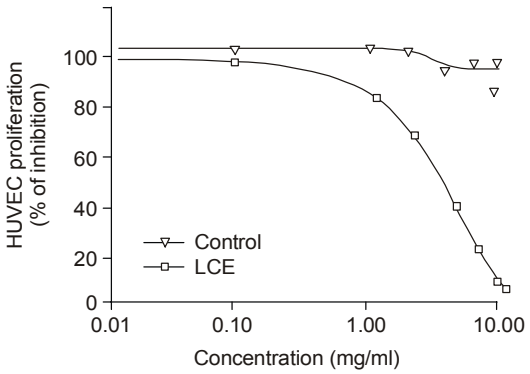


Fig. 3. Ingibition of endothelial cell proliferation by LCE.

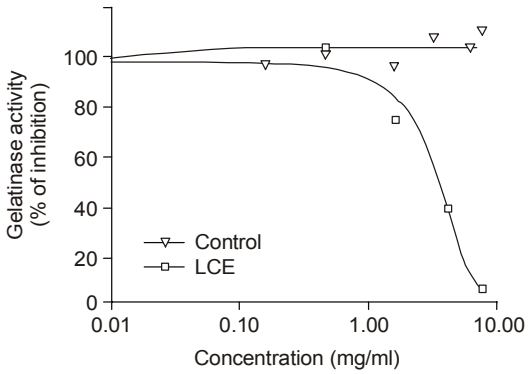


Fig. 4. Inhibition of gelatinolytic activity (MMP-2) by LCE.

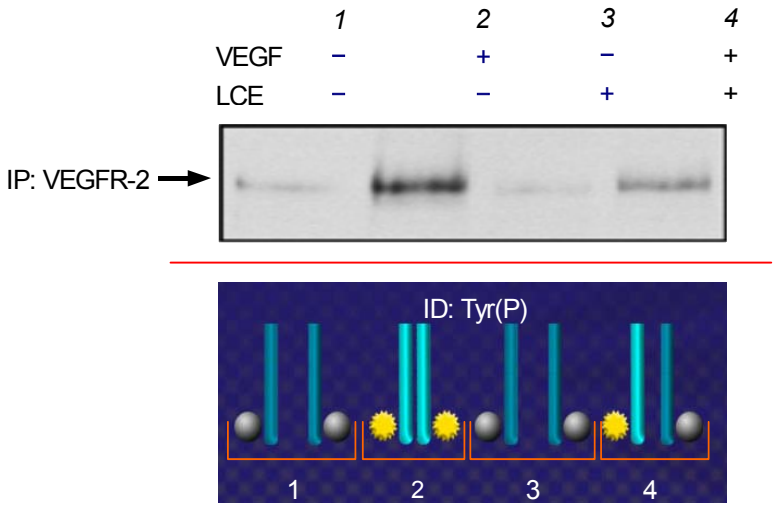


Fig. 5. Inhibition of VEGF-dependent VEGF tyrosine phosphorylation by LCE.

this class of enzymes. The inhibitory effect of LCE towards the gelatinolytic activity of MMP-2 was confirmed by zymography (Fig. 4). Zymography is a versatile two-stage technique involving protein separation by gel electrophoresis followed by detection of proteolytic activity toward a known substrate imbedded in the gel. Incubation of the zymogram with increasing concentrations of LCE resulted a dose-dependent inhibition of the gelatinolytic activity of MMP-2. Inhibition was almost completed at a concentration of 10 mg/ml while a negative control was without any effect.

Inhibition of VEGF Receptor Activity

As mentioned earlier, vascular growth factor (VEGF) is a potent trigger of angiogenesis. Endothelial cells express VEGF receptors which are activated by tyrosine phosphorylation upon binding of the factor. Phosphorylation of the receptors results in a cascade of signaling events inside the cell, leading to angiogenic activities. To further investigate the anti-angiogenic potential of LCE, VEGF-dependent tyrosine phosphorylation of VEGFR-2 was measured on bovine endothelial cells (BAEC) in

the presence and absence of the cartilage extract. As shown here (Fig. 5), LCE was able to significantly inhibit VEGF-induced receptor phosphorylation signal, thus supporting its anti-angiogenic potential.

Chemotherapy

Chemotherapy, used alone or in combination with other standard therapies, is the tool of choice to slow down the evolution of most cancers. A successful chemotherapy will track and specifically kill all cancer cells of one's organism. Unfortunately this therapeutic ideal is seldom attained because cancer cells naturally have certain means to resist the action of chemotherapeutic agents (Links et Brown, 1999).

Chemotherapy and Drug Resistance

In fact, cancer cells generally react well to a first round of chemotherapy. Their number decreases to a level where their presence cannot be detected anymore and the patient is then considered in remission. This more or less long calm spell unfortunately is still too often followed by a relapse (Fig. 6). Some cancerous cells more resistant than average can indeed survive the first offensive of chemotherapy. Being very few, they are not easily detectable up to the moment when, having recovered from chemotherapy insults, they start dividing at a fast pace. One notes in clinic that these surviving cells respond in general little or not at all the subsequent treatments of chemotherapy. Reapplying the initial therapeutic protocol turns out to be ineffective on these cells and increasing doses may exacerbate side effects to an unacceptable level. Even opting for a different arsenal of chemotherapeutic agents may not make it possible to break this resistance. The cancer cells have now develop resistance to multiple chemically and functionally unrelated anti-tumor compounds (Ford, 1996). This phenomenon is called multi-drug resistance.

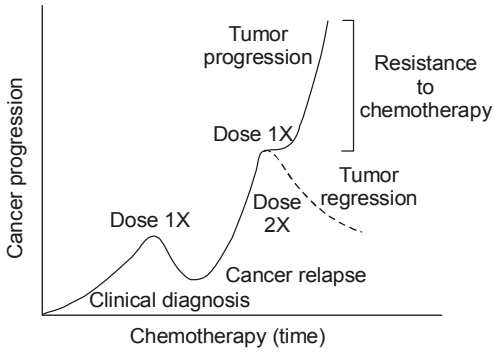


Fig. 6. Chemotherapy and drug resistance.

What is the Molecular Mechanism Underlying This Drug Resistance?

A major reason for such chemotherapeutic agent resistance is the presence of proteins called P-gp within the membrane of the cancerous cells (Lehne G, 2000). P-gp proteins are pumps that actively extrude the activated chemotherapeutic agents from the interior of the cancer cells to the extracellular

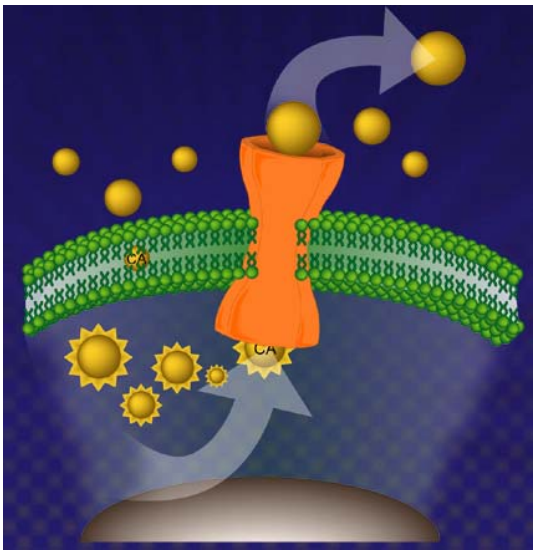


Fig. 7. Extrusion of chemotherapeutic agents through P-gp pump.

space where they can no longer exert their anti-tumour action (Fig. 7). By preventing accumulation of chemotherapeutic agents inside the cancer cells, P-gp pumps action seriously impairs the efficacy of treatments (Shustik et al, 1995). Moreover, this pump is not very selective and will expel the vast majority of chemotherapeutic agents.

One of the underhand effects of chemotherapy is the selection for survival of cancerous cells over-expressing P-gp from an initially heterogeneous population for P-gp expression (Bosch et Croop, 1996. The cells overexpressing P-gp pumps indeed manage to survive the initial chemotherapy to possibly form a new clonal population of cancerous cells from now on refractory to most chemotherapeutic agents; chemotherapy resistance is established.

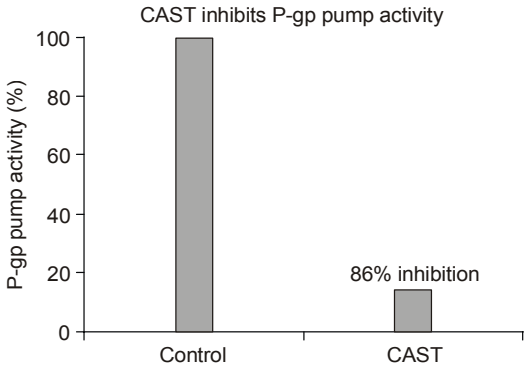


Fig. 8. Inhibitory action of CAST on P-gp pump activity.

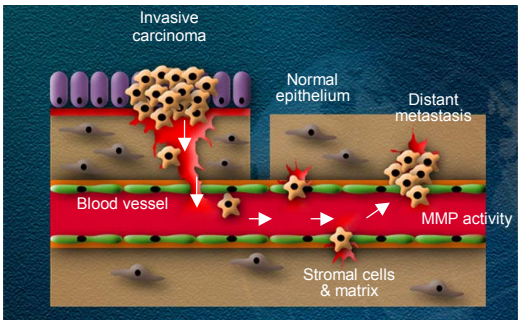
CAST Biological Actions

CAST (Chemotherapy Active Support Technology) is a multivalent all-natural formula acting as a chemosensitizer and an inhibitor of metastasis as well. Cast ingredients were judiciously selected for their synergistic action in order to achieve an optimal support to chemotherapy. Inclusion of Atrium's proprietary AOMC ingredient makes CAST a unique formula. Moreover, the various levels of solubility of CAST ingredients makes it possible to saturate simultaneously both aqueous and lipidic pools of the human body. This ensures an excellent bioavailability for CAST.

CAST Chemosensitizing Activity

The main strength of CAST resides in its ability to inhibit P-gp pumps activity in cells. A scientific study carried out *in vitro*, by an independent laboratory, showed an inhibition of nearly 86% of the activity of P-gp pumps in the presence of CAST (Fig. 8).

Based on these convincing results, CAST is expected to act as a valuable therapeutic support. By inhibiting P-gp pumps activity, CAST allows chemotherapeutic agents to accumulate within tumor cells to better exercise their anti-tumor action. In fact, not only should CAST preserve the sensitivity



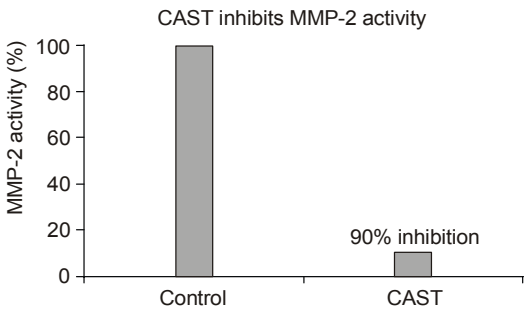


Fig. 9. MMP activity during metastasis and the inhibitory action of CAST on MMP action.

of the cancer cells towards chemotherapeutic agents, such as vincristine, doxorubicin, vinblastine and etoposide (among others) but it is expected to even restore it where lost, such as in chemotherapy refractory cancer cells (Sikic et al, 1997).

CAST Inhibition of MMP Activity

However, sensitizing cancerous cells to chemotherapeutic drugs is not the only mode of action of CAST. This multipotent product also has the potential to help prevent cancer dissemination by antagonizing the action of some metalloproteinases (MMPs), as shown in an in vitro assay for MMP-2 activity (Fig. 9).

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases, which can degrade the major components of the extracellular matrix (ECM) (Fig. 9). Cancer cells subvert MMPs activity to promote invasion of the surrounding tissues as well as metastasis to distant ones. MMPs, by releasing growth factors sequestered in the extracellular matrix, also are thought to promote the growth of these tumor cells once they have metastasized (Chang et Werb, 2001). Thus by opposing MMP-2 activity, CAST shall prevent tumor progression and metastatis.

CONCLUSION

1) LCE and CAST as natural inhibitors of tumor-related pathways

Angiogenesis induction and Pg-p mediated chemotherapy resistance figure as main weapons within the tumor armamentarium that is put forward to escape natural body's defenses. Angiogenesis is itself a complex phenomenon that involves the interplay of MMP activity, endothelial cell proliferation and VEGF signaling. On the other hand, the presence of several pathways controlling angiogenesis are as many targets in the development of modulating agents.

The LCE have demonstrated numerous biological activities that are in relation with the angiogenesis process. It has been shown that LCE inhibits MMP enzymatic activity, endothelial cell proliferation and VEGF-induced pro-angiogenic signaling through receptor activation. The resulting action of LCE would reside in the starving of tumors and prevent both, tumor progression and metastasis formation.

The strength of CAST is in its multivalent approach to support cancer treatment. First it inhibits the activity of the P-gp pumps on cancer cells. Knocking down this resistance mechanism maintains the chemotherapeutic drugs within the tumor cells where they can fully express their anti-tumor action. This ensures that every tumor cells receives its due dose of chemotherapeutic drugs regardless of how many P-gp pumps it may express. Making all tumor cells equals in the face of therapy may prevent the appearance of chemotherapy agent resistance. Second, inhibiting the proteolytic activity of MMP-2 on the collagen matrix prevents the switch to a more invasive type of cancer. Maintaining the cancer cells sensitivity to therapeutic agents and preventing the growth of tumors and the development of metastasis shall greatly improve the overall rate of survival among cancer patients under a chemotherapeutic regimen.

(1) The combination of LCE and CAST as a clinical regimen allied: LCE and CAST can be used to supplement the diet and support standard therapies. For instance, at diagnosis, LCE can be used to slow down angiogenesis while CAST can increase the effectiveness of a standard chemotherapy protocol by acting on P-gp pumps. Also, when the clinician has to deal with a relapse situation where chemotherapeutic agent resistance is already established, CAST as well as LCE can and prevent.

REFERENCES

1) Berbari P, Thibodeau A, Germain L, Saint-Cyr M,

Gaudreau P, El-Khouri S, Dupont E, Garrel DR. Antiangiogenic effects of the oral administration of liquid cartilage extract in humans. *J Surg Res* 1999; 87(1): 108-13.

2) Bosch I, Croop J. P-glycoprotein multidrug resistance and cancer. *Biochim Biophys Acta* 1996; 1288(2): F37-54

3) Chang C, Werb Z. The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. Trends Cell Biol 2001; 11(11): S37-43.

4) Ford JM. Experimental reversal of P-glycoprotein-mediated multidrug resistance by pharmacological chemosensitisers. *Eur J Cancer* 1996; 32A: 991-1001

5) Lehne G. P-glycoprotein as a drug target in the treatment of multidrug resistant cancer. *Curr Drug Targets* 2000; 1(1): 85-99

6) Links M, Brown R. Clinical relevance of the molecular mechanisms of resistance to anti- cancer drugs. Expert Reviews in Molecular Medicine. Cambridge University Press. 1999

7) Shustik C, Dalton W, Gros P. P-glycoprotein-mediated multidrug resistance in tumor cells: biochemistry, clinical relevance and modulation. *Molec Aspects Med* 1995; 16: 1-78.

8) Sikic BI, Fisher GA, Lum BL, Halsey J, Beketic-Oreskovic L, Chen G. Modulation and prevention of multidrug resistance by inhibitors of P-glycoprotein. *Cancer Chemother Pharmacol* 1997; 40 Suppl: S13-9

9) Zetter BR. Angiogenesis and tumor metastasis. *Annu Rev Med* 1998; 49: 407-24.