Angiogenesis and P-glycoprotein: Their Roles in Cancer
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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 having to cope with the circumstances. However, thanks to modern medicine 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 to awareness of healthier practices of life includes balanced nutrition to help maintain the normal body’s homeostasis.

At the cellular level, there are strategies by which a cancer can encourage its growth or even escape treatment attempts. Angiogenesis, the formation of new blood vessels, is induced by tumors and serves to direct vital blood nutrients and oxygen to the tumor itself to support its growth. Blood vessel proximity also represents a way out through which tumor cells can escape from the mother tumor and disseminate to distant organs and colonize as daughter metastases. However, while feeding on nutrient substances conveyed by blood vessels, the tumor is also able to activate special devices to eliminate molecules representing a threat to its own survival. P-glycoprotein (P-gp) pumps are part of this defense mechanism that cancers use to extrude chemotherapeutic agents (CA) out of tumor cells. P-gp action eventually leads to CA resistance and thus seriously hampers chemotherapy efficacy.

This paper will discuss the roles of angiogenesis and P-gp in tumor growth. It will also describe recent advances in nutrition technology that show promise in modulating angiogenesis, Matrix Metalloproteinase (MMPs) action and P-gp activities.

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

Various triggering events (pro-angiogenic 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 represents a key factor in the promotion of tumor angiogenesis.

During the initial phase of their activation process, endothelial cells will secrete specialized enzymes known as matrix metalloproteinases (MMPs). These MMPs are required to digest the collagen fibers present in the basement membrane of blood vessels. This creates a breach through which proliferating endothelial cells can migrate in the direction of the pro-angiogenic signal source (step II). Along their path, endothelial cells produce an extracellular matrix that assembles 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 (Figure 2).

Formation of new blood vessels brings oxygen and nutrients to the microscopic tumor cell mass (Figure 2, left, Initiation step). This leads to tumor cell proliferation and primary tumor growth to clinically palpable sizes. Angiogenesis also allows for systemic tumor dissemination.

Figure 1: Angiogenesis: A Complex Process

- Cells derived of oxygen and angiogenic signal
- Inflammatory signal cells
- Re构建 cells

- Endothelial cell migration and proliferation host anastomosis
- Signal source
- Functional vasculature
- Oxygen and nutrient supply

- A new blood vessel is formed

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Individual tumor cells may break out from 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 repeated 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 (Figure 2). Indeed, blocking the progression of new blood vessel 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 (liquid cartilage extract) 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 action of the pro-angiogenic factor VEGF. LCE is obtained through sophisticated ultra-filtration steps that are part of an Atrium patented manufacturing process. More importantly, the oral bioavailability of LCE has been 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 of MMP-2 Activity

MMP activity also being important for angiogenesis, we sought for a potential effect of LCE on the enzymatic activity of MMP-2, a representative of this class of enzymes. The inhibitory effect of LCE towards the gelatinolytic activity of MMP-2 was confirmed using a fluorescence-based in vitro assay (Fig. 4). In this assay, a purified MMP-2 enzyme is incubated in the presence of gelatin as a substrate and various concentrations of LCE. The inhibition of the MMP-2 activity can be measured through the reading of the emitted fluorescence. This experiment demonstrates that LCE resulted in a dose-dependent inhibition of the degradation activity of MMP-2.

Inhibition of VEGF Receptor Activation

As mentioned earlier, vascular endothelial growth factor (VEGF) is a potent trigger of angiogenesis. Endothelial cells express VEGF receptors which are activated by tyrosine (Tyr) phosphorylation (P) 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 endothelial cells in the presence and absence of the cartilage extract. As shown here (Fig. 5, lane 4), LCE was able to significantly inhibit VEGF-induced receptor phosphorylation signal, thus supporting its anti-angiogenic potential.

Chemotherapy: A Clinical Weapon Against Tumor Growth

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. 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).
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Chemotherapy and 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 to 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 developed resistance to multiple chemically and functionally unrelated anti-tumor compounds (Ford, 1996).

This phenomenon is called multi-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 space where they can no longer exert their anti-tumor 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 encountered.

One of the devious effects of chemotherapy is the selection for survival of cancerous cells overexpressing 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, now refractory to most chemotherapeutic agents; chemotherapy resistance is established.

CAST Biological Actions
CAST (Chemotherapy Active Support Technology) acts as a chemosensitizer and an inhibitor of metastases as well. CAST ingredients were judiciously selected for their synergistic action in order to achieve an optimal support to chemotherapy. Moreover, the various levels of solubility of CAST ingredients makes it possible to saturate simultaneously both aqueous and lipidic pools of the human body.

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 showed an inhibition of nearly 86% of the activity of P-gp pumps in the presence of CAST (Fig. 8). It was also shown that this action leads to the increase of the intracellular accumulation of chemotherapeutic agent (CA) in tumor cells.

Figure 3: Inhibition of Endothelial Cell Proliferation by LCE

Figure 4: Inhibition of Gelatinolytic Activity (MMP-2) by LCE
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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 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 tumors (Sikic et al, 1997).

CAST Inhibition of MMP Activity

Sensitizing tumor cells to chemotherapeutic agents 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, 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). Cancer cells subvert MMPs' activity to promote invasion of the surrounding tissues as well as metastases to distant ones (Fig. 9). 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 metastasis.

Conclusion

The Combination of LCE and CAST as a Clinical Regimen Naturally Allied

Angiogenesis induction and Pg-p-mediated chemotherapy resistance figure as main weapons within the tumor armamentarium that is put forward to escape natural body defenses as well as clinical aids. Angiogenesis is itself a complex phenomenon that involves the interplay of MMP activity, endothelial cell proliferation and VEGF signaling.

The LCE have demonstrated numerous biological activities that are related to 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 metastases formation. Furthermore, the bioavailability of the antiangiogenic activity of LCE has been demonstrated in humans.

The strength of CAST is in its multivalent approach to support cancer treatment. First it inhibits the activity of the P-gp pumps. Knocking down this resistance mechanism maintains the chemotherapeutic agents within the tumor cells where they can fully express their anti-tumor action. This ensures that every tumor cell receives its due dose of chemotherapeutic agents regardless of how many P-gp pumps it may express. Making all tumor cells equal in the face of chemotherapy may further prevent the appearance of chemotherapy 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 metastases shall greatly improve the overall rate of survival among cancer patients under a chemotherapeutic regimen.

Taken together, LCE and CAST, may well represent synergistic dietary supplementation to help support standard cancer therapies.

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Bibliography


