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COLLAGEN AND TUMORS

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Abstract

The cancer derives from alterations of different genes that lead to greater aggressiveness of the initial tumor, in a progressive occurrence of events. Several stages are necessary, a kind of "pentathlon" for the metastases to form. The migration of the "restless" cells contributes to the push of the primary tumor environment that surrounds them, which becomes inhospitable as the tumor grows, so that some more enterprising elements come off in search of a new location, find it, colonize it and they multiply. To do this they must cross the body's tissues that act as a barrier. Several studies have allowed us to understand the role of metalloproteinases in the development of tumor metastases, such as enzymes responsible for the degradation of the matrix in particular of collagen. The amount of experimental and clinical evidence that associates MMPs with tumor progression has prompted Jews to synthesize compounds targeting MMPs to be tested in clinical trials as MMP inhibitors (MMPIs) for various types of cancer types. Extracellular matrix (ECM) is not only a mechanical support for cells, but it represents a veritable reservoir of growth factors, with phenotypic consequences in cellular behaviour, and intervenes in regulating intercellular communication, both in physiological conditions (proliferation, differentiation, apoptosis) and in pathological ones, for example in the development of tumor metastases. On the same basis we could hypothesize the experimentation of the intake of dietary supplements for the prevention of tumor metastases.

Keywords: *metastasis, metalloproteinase, collagen, extracellular matrix*

Introduction

The extracellular matrix (ECM) is a complex network of extracellular-secreted macromolecules, such as collagen, enzymes and glycoproteins, whose main functions deal with structural scaffolding and biochemical support of cells and tissues. ECM homeostasis is essential for organ development and functioning under physiological conditions, while its sustained modification or dysregulation can result in pathological conditions (1).

The extracellular matrix is composed of glycosaminoglycans (GAGs), proteoglycans, adhesive glycoproteins, and structural fibrous proteins. Two types of extra-cellular matrix can be distinguished: the extracellular interstitial matrix (ECM) and the basement membrane associated with the epithelium (BM). Some tissues, such as the epithelial one, are made up of cell sheets with very little extracellular matrix, instead the connective tissue is mainly composed of the extracellular matrix with scattered cells. The components of the extracellular matrix are synthesized by the resident cells and then secreted by exocytosis, then each cell synthesizes specific molecules in specific tissues. GAGs are linear polysaccharide molecules, long chains formed by the repetition of a disaccharide. Proteoglycans are a family of macromolecules made up of a protein core to which they covalently bind to the GAGs, which make them able to attract internally large quantities of water, giving the matrix considerable resistance to compression and delaying the spread of pathogens of various nature, as well as metastatic cells. The proteoglycans in association with the basal lamina form a sort of selective molecular filter for the macromolecules. The adhesive glycoproteins have numerous domains to interact with integrins, cell surface proteins, and with the components of the extracellular matrix (collagen fibers and proteoglycans), thus facilitating the adhesion of cells to the matrix. The main glycoproteins are fibronectin, laminin, entactin, tenascine, chondronectin and osteonectin. The ability of the extracellular matrix to resist compression is ensured by the presence of proteoglycans and GAGs that keep it hydrated while the tensile strength is a consequence of the presence of inextensible collagen fibers. Collagen fibers, which make up about 20% of all body proteins, are composed of tropocollagen subunits. Each single tropocollagen molecule is formed by three polypeptide chains, α chains, wrapped together in a triple helix configuration. These molecules tend to assemble spontaneously forming collagen fibrils, which in turn aggregate to give rise to collagen fibers. Each α chain is made up of 1000 amino acids, a third of which is represented by glycine which is repeated every three residues, while the remainder is represented mainly by proline, hydroxyproline and hydroxylisine. Glycine is a very small amino acid, thanks to its structure it allows the helical folding of the α chains and their compaction. Hydrogen bonds, which are formed with the participation of hydroxyproline, keep the three α chains together, while hydroxylisine plays a role in keeping tropocollagen molecules together (2). There are different types of collagen that are located in specific regions of the body where they perform equally specific roles. Type I collagen is the most common, forming fairly thick fibers in the connective tissue itself, in bone tissue, dentin and concrete. It is synthesized from fibroblasts, osteoblasts, odontoblasts and cementoblasts. Type II collagen forms thinner fibers and is almost exclusive to hyaline and elastic cartilage, it is synthesized by chondroblasts. Type III collagen is a type of highly glycosylated collagen, it is synthesized from fibroblasts, reticular cells, smooth muscle cells and hepatocytes. This type of collagen is found in the lymphatic system, spleen, liver, cardiovascular system, lungs, skin. Type IV collagen does not form fibrils, but networks of procollagen molecules that combine to form a framework for the basement membrane. It is synthesized from epithelial, muscular and Schwann cells. Type V collagen forms very thin fibrils usually associated with type I collagen. Their synthesis is carried out by mesenchymal cells and fibroblasts. Type VI collagen forms dimers which are assembled into specialized structures, and which help to connect the basal lamina of the multilayered epithelia to the underlying connective tissue. It is located in the interstitial space and is associated with type I collagen. Type VII collagen forms small aggregates, anchoring fibrils, which ensure the adhesion of the basement membrane to the underlying fibers of type I and II. It is synthesized by epidermal cells and is found in the junctions of the epidermis and

dermis. Several types of proteolytic enzymes are involved in matrix degradation and among these the main group is that of serine proteases, cysteine proteases and MMPs (3,4,5).

Methods

The ECM should not only be considered as a mechanical support for cells, but as a real reservoir of growth factors and its possible remodeling can have phenotypic consequences in cellular behaviour. It also plays a very important role in regulating intercellular communication, both in physiological conditions (proliferation, differentiation, apoptosis) or in pathological conditions, for example in the development of tumor metastases. The cancer derives from alterations of different genes that lead to a greater aggressiveness of the starting tumor, in a progressive occurrence of events. Several stages are necessary, a kind of "pentathlon" for the metastases to form. The migration of the "restless" cells contributes to the push of the primary tumor environment that surrounds them, which becomes inhospitable as the tumor grows, so that some more enterprising elements come off in search of a new location, find it, colonize it and they multiply. To do this they must cross the body's tissues that act as a barrier.

During the advancement of cancer, epithelial tumor cells may undergo epithelial-to-mesenchymal transition (EMT), a morphological and functional remodeling, that deeply alters tumor cell features. The loss of epithelial markers occurs (i.e., E-cadherin), with associated changes in cell polarity and intercellular junctions, increasing of mesenchymal markers (i.e., N-cadherin, fibronectin and vimentin). In this way original tumor degenerates, thus allowing cancer cells to enter the lymphatic flow and colonize distant sites. This type of migration is still obscure, but some modifications occurring in target tissue ECM. Matrix metalloproteases and several adhesion receptors, among which integrins play a key role, are involved in metastasis-linked ECM modifications. promoting cancer cells adhesion and growth. Tumor cell plasticity during all the above steps is supported by two main mechanisms: epithelial to mesenchymal transition (EMT) and its reverse counterpart mesenchymal to epithelial transition (MET).The EMT process has been recognized as a biological cell reprogramming characterized by loss of cell adhesion, inhibition of E-cadherin expression and increased cell mobility. EMT is essential during development and is regulated by several transcription factors, many of which are actually considered EMT markers: zinc finger protein snail 1 (SNAI1, SNAI2), zinc finger E-box-binding homeobox 1 (ZEB1, ZEB2), TWIST, forkhead box protein 1 (FOXC1), FOXC2, homeobox protein goosecoid (GSC), N-cadherin, vimentin and fibronectin-1. However, EMT and MET processes, though extensively studied, are still far from being completely understood; the prevailing hypothesis claims that EMT operates in the first steps to form CTCs that, upon survival in the circulation, after reaching the appropriate organ site, undergo MET thus gaining appropriate features required to prepare the soil as premetastatic niche and promote tumor progression.

Tumor cells, CAFs, myofibroblasts, macrophages and other stromal components promote cancer cell invasion by secreting MMPs that, in turn, lead to ECM degradation, a necessary step to allow cancer cell invasion.

Results

The MMP-mediated ECM modifications influence tumor progression by facilitating the growth of solid tumor mass with different mechanisms. MMPs are zinc-dependent ECM remodeling endopeptidases involved in all steps of metastasis and a high MMP expression in tumors correlates with poor prognosis and recurrence. Denaturation of fibrillar collagen stimulates melanoma cell proliferation through down-regulation of p27kip1. MMPs can contribute to the release ECM-embedded growth factors, for example VEGF plays a key role in vascular homeostasis with the proliferation of surrounding cells, the formation of new blood vessels, enhancing vascular permeability. The ADAM (a disintegrin and metalloproteinases) family members, including ADAM8, ADAM9, ADAM10, ADAM12 and ADAM15, exert their action by degrading the ECM proteins collagen IV and FN, thus contributing to niche formation and cancer progression. ADAM8, besides cleaving important ECM components of the tumor

stroma such as collagen I, fibronectin and periostin, can cluster with 1 integrin and could direct tumor cell invasion through localized proteolytic ECM degradation in protrusions of cancer cells. Meprins as enzymes cleave ECM proteins such as collagen IV and FN and can also indirectly regulate ECM remodeling by activating other metalloproteinases. Meprin is expressed in several different tumors. For these reasons, meprin is thought to alter ECM components structure, thereby aecting proliferation and migration of tumor cells into the surrounding tissue. Another family of MMPs implicated in cancer progression is represented by bone morphogenetic protein (BPM)/tolloid-like proteinases (BTPs). These enzymes are involved in the maturation of procollagens I–III, of fibrillar collagens V and XI and cleave procollagens V and XI.

Discussion

The amount of experimental and clinical evidence associating MMPs with tumor progression prompted efforts in synthesizing compounds targeting MMPs to be tested in clinical trials as MMP inhibitors (MMPIs) for various cancer types. In spite of the large efforts made, MMPIs uniformly failed to demonstrate a survival benefit and, more importantly, severe side effects were reported. The reasons for this failure are still debated and probably administration of MMPIs in the early cancer stages rather than in stage IV could open new perspectives. However, now no tangible prospect of clinical use for MMPIs are in sight. MMPs catalyze their own neutral pH substrates, have a multi-domain structure formed by: a pro-domain, a catalytic domain, a hinge region and a hemopexin-type motif. The signal peptide directs the proteases through the secretory pathway of the cell and the prodominio maintains these enzymes in an inactive form. The catalytic domain contains: the binding site for Zn^{+2}, three histidine residues and a methionine that forms a "Met-turn" eight downstream residues. The "Met-tum" is necessary to support the "dimple" structure around the Zn^{+2}. The hinge region called "hinge", about 75 amino acids long, is rich in proline and binds the catalytic domain to the C-terminal "hemopexin-like" domain. This last domain (about 200 amino acids) determines the substrate specificity of the matrix metalloproteases and mediates the interaction with the endogenous tissue inhibitors TIMP (tissue inhibitors of metalloproteinases). The matrix metalloproteases are maintained in a catalytically inactive state by the interaction between the thiol group of the cysteine residue (Cys73) of the prodominio and the zinc ion in the catalytic site. To be activated, they need the breakdown of this interaction in a process called "Cysteine switch".

These are therefore topics that are still being studied in order to be able to talk about curative cancer therapy or metastasis arrest, to base some studies also on the preventive use of dietary supplements. According to the new European Code against cancer, there are 12 suggestions based on the best scientific evidence available to adopt healthy lifestyles and to support anticancer prevention in everyday life (6):

1 Do not smoke. Do not use tobacco

2 No smoking in the house. Supports workplace anti-smoking policies

3 Make sure you keep your body weight healthy

4 Be physically active every day. Limit the time you spend sitting

5 Follow a healthy diet: mainly eat whole grains, legumes, vegetables and fruit limits high-calorie foods (foods with high sugar and fat content) and avoid sugary drinks and preserved meat; limits red meat and foods that are high in salt, If you drink alcohol, limit your intake. Drinking alcohol is not recommended for cancer prevention

6 Avoid long exposure to the sun, with particular attention to children. Use sunscreens. Do not use solar lamps

7 At work, protect yourself from exposure to carcinogens by following the safety instructions

8 Check if you are exposed to high levels of radon radiation at home. Activated to reduce radon exposure levels

9 For women: breastfeeding reduces the risk of cancer in women. If you can, breastfeed your baby.

10 Hormone replacement therapy (HRT) increases the risk of certain types of cancer. Limit the use of HRT

11 Make sure your child is vaccinated for: Hepatitis B (for newborns) Papillomavirus - HPV (for girls)

12 Join screening programs for: bowel cancer (men and women), breast cancer (women), cervical cancer (women).

In this case we are talking about supplements that aim to help the maintenance of ECM homeostasis, in particular aimed at the good functioning of collagen and MMPs. The body produces its own collagen every day, but as production decreases with age, the available supply of collagen quickly becomes insufficient and the different parts of the body gradually deteriorate. Hydroxyproline, which is critical for collagen stability, is synthesized by hydroxylation of the amino acid proline. The reaction requires organic silicon and vitamin C to allow the addition of oxygen. It acts as a metabolic protector, acting at various levels: it opposes the lipid peroxidation responsible for freeing free radicals, counteracts the non-enzymatic cross-linking and glycosylation of the constituent proteins of the connective tissue that cause stiffness and sclerosis. It regulates and stimulates fibroblast mitoses and for this property plays a fundamental role in the regeneration process of dermal and epidermal cells. It is a co-enzyme of prolin hydroxylase essential for the endogenous synthesis of hydroxyprolin. White bread, shelled seeds and peeled fruit lose their silicon content, thus facilitating the accumulation of calcium in the arteries and articular cartilage. The exogenous supplementation of silicon in the diet allows, through the normalization of the concentration of orthosilicic acid, to regulate the formation of the extracellular matrix and the calcium metabolism. Silicon is a structural element of the connective tissue and enters the constitution of the main macromolecules such as elastin, collagen, proteoglycans and glycoproteins, promoting their regeneration. Metalloproteinases (MMPs) regulate the degradation of collagen as a function of mechanical loading. There are also other factors that intensify the loss of collagen, such as excessive use of the osteoarticular-muscular system (intense physical activity), trauma, menopause. Overweight, cancer and cancer treatments, cortisone, smoke inflammation, sedentary lifestyle. The metal-proteins govern the penetration of tumor cells into tissues and vessels and are inhibited in vitro by: Gallate of epigallocatechin, curcumin, quercetin, resveratrol, genistein, vitamin C, vitamin E, OPC (procyanidine oligomer from *Pinus maritima* and *Vitis vinifera*). Therefore it is advisable:

-Integration of hydrolyzed collagen of porcine, or marine origin
-Integration of silicon from bamboo, horsetail or algae: to increase collagen synthesis)
-Vitamin C: to activate the synthesis of hydroxyproline and collagen
-Integration of MSM (Methyl Sulfonyl Methane): to elasticize the collagen
-OPC or oligoproanthocianidins (peel of red grapes and *Pinus maritima*) and anthocyanins (berries and cranberry): to stabilize collagen (inhibit the metalloproteasis)
-MSM (methyl sulfonyl methane) restores the formation of cross-links in collagen preserving its elasticity; it is considered a synergistic element in the stimulus to collagen synthesis for the amino acids cystine, glycine, hydroxyproline, ornithine alpha keto glutarate and silicon. MSM is also a powerful antioxidant, able to hinder and deactivate the action of free radicals
-astaxanthin combined with collagen hydrolysate improves facial elasticity and decreases matrix metalloproteinase-1 and-12 expression. In a clinical study on a total of 44 healthy subjects recruited and treated with astaxanthin (2 mg/day) combined with collagen hydrolysate (3 g/day) or placebos, authors reported an improved elasticity and barrier integrity in photoaged facial skin, and such treatment is well tolerated (7).
Zangue and coauthors suggests that daily ingestion of collagen hydrolysate CH may reduce aging-related changes of the extracellular matrix by stimulating anabolic processes in skin tissue (8).

Another study reports oral collagen peptide supplementation significantly increased skin hydration after 8 weeks of intake. The collagen density in the dermis significantly increased and the fragmentation of the dermal collagen network significantly decreased already after 4 weeks of supplementation (9).

But to exert its power collagen must be optimally absorbed. One study reports the postprandial absorption of collagen. A randomized, blinded, cross-over study was conducted in which ten healthy male subjects received 35 g of enzymatically hydrolyzed collagen (EHC), 35 g of non-enzymatic hydrolyzed collagen (NC) or placebo (250 mL of water) in three non-consecutive days. The absorption rate and the bioavailability of glycine, proline and hydroxyproline were significantly higher for EHC ($p < 0.05$) (10).

What we do know is that there are no side effects, that the use of these supplements is confirmed with regards to aging, muscle strength, improvement of osteoarticular pathologies, with regard to tumors we await further studies.

References

1. Amălinei, C., Căruntu, I. D., & Bălan, R. A. (2007). Biology of metalloproteinases. *Rom J Morphol Embryol*, *48*(4), 323-334.
2. Li, P., & Wu, G. (2018). Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. *Amino acids*, *50*(1), 29-38.
3. Parks, W. C., Wilson, C. L., & López-Boado, Y. S. (2004). Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nature Reviews Immunology*, *4*(8), 617-629.
4. Page-McCaw A., Ewald A.J., Werb Z., *Matrix metalloproteinases and the regulation of tissue remodeling*, Nature Reviews Molecular Cell Biology, 2007, 8: 221–233
5. Pickup, M. W., Mouw, J. K., & Weaver, V. M. (2014). The extracellular matrix modulates the hallmarks of cancer. *EMBO reports*, *15*(12), 1243-1253.
6. https://www.airc.it/cancro/prevenzione-tumore/prevenzione-per-tutti/codice-europeo-contro-il-cancro
7. Yoon, H. S., Cho, H. H., Cho, S., Lee, S. R., Shin, M. H., & Chung, J. H. (2014). Supplementing with dietary astaxanthin combined with collagen hydrolysate improves facial elasticity and decreases matrix metalloproteinase-1 and-12 expression: a comparative study with placebo. *Journal of medicinal food*, *17*(7), 810-816.
8. Zague, V., de Freitas, V., Rosa, M. D. C., de Castro, G. A., Jaeger, R. G., & Machado-Santelli, G. M. (2011). Collagen hydrolysate intake increases skin collagen expression and suppresses matrix metalloproteinase 2 activity. *Journal of medicinal food*, *14*(6), 618-624.
9. Asserin, J., Lati, E., Shioya, T., & Prawitt, J. (2015). The effect of oral collagen peptide supplementation on skin moisture and the dermal collagen network: evidence from an ex vivo model and randomized, placebo-controlled clinical trials. Journal of cosmetic dermatology, 14(4), 291-301.
10. Skov, K., Oxfeldt, M., Thøgersen, R., Hansen, M., & Bertram, H. C. (2019). Enzymatic Hydrolysis of a Collagen Hydrolysate Enhances Postprandial Absorption Rate—A Randomized Controlled Trial. Nutrients, 11(5), 1064.)