ORIGINALIA

L. Turco

THE MATRIX TETRAMETRIC CODE: HORMONES, CYTOKINES, NEUROPEPTIDES, MELATONIN

SUMMARY

The scavenger cell system plays a key role in the local and systemic inflammatory response, in the recognition and processing of non-self stimulus and in biological regulation. These activities require a changeable extracellular area and prompt microenvironment information adaptation. Matrix remodelling is a process common to a variety of physiological and pathological conditions (traumas, infections, injuries, atherosclerosis, etc.) and it is the main trigger for regulatory responses.

Recent evidence shows that metalloproteinases mediate the remodelling processes, thereby supporting the capillary-matrix-receptor morphofunctional unit modulation.

- "Low dilutions" of HCNM (hormones, cytokines, neuropeptides, melatonin) contribute to MMPs/TIMPs balancing, and codify - at the same time - matrix flexibility and order PGs-GAGs net local memory. Moreover, matrix plasticity refers to a group of learning algorithms in fuzzy signals.

PAROLE CHIAVE

REGULATION, MATRIXIN, REMODELLING, MATRIX CLOCK.

INTRODUCTION

The clock of *Physiological Regulation* is calibrated according to a never-ending series of metabolic stimuli, its unbroken trend being ensured by changing schemes and adaptation responses determining the continuity and the discontinuity of physiological processes.

The activity of **metalloproteases** is calibrated in the matrix using the latent forms of proenzyme and/or the action of endogenous inhibitors (TIMPs) through hormone, cytokine, neuropeptide and melatonin-mediated modulation (HCNM).

- Matrix *remodelling* and *inhibition* are processes contributing to metabolism: a peculiar characteristic of the first process is the plasticity and dynamism of response to internal and external changes; while the inhibition process is characterized by a special harmony between the elements of which it is composed.

The aim of the matrix changes (metabolè) is an alternation between continuity and change which occurs throughout the life cycle.

MATRIX METALLOPROTEASES

The matrix metalloproteases (MMPs) are an enzyme family involved in the remodelling processes of the extracellular matrix (ECM) and are related to both mental and physiological events, such as inflammation, cancer invasiveness, growth and metabolism.

The therapeutic imitation and application of the physiological changes of the matrix proteases is a target for the *physiological modelling* of the extracellular space.

After identifying the first collagenase on a changing tadpole tail, 28 different matrix metalloproteases or matrixins have been identified in the vertebrata (22).

MMPs are usually classified into 6 groups (21, 25, 35, 37) (FIGURE 1):

1. interstitial collagenases (MMP-1, -8 and -13);
2. stromelysins (MMP-3, -10, -11 and -12);
3. matrilysin (MMP-7 and -26);
4. gelatinase (MMP-2 and -9);
5. membrane MMPs (MMP-14, -15, -16, -17, -24 and -25);
6. other MMPs that are not included in the groups mentioned above (MMP-19, -23 and -28).

The most studied matrixins (*page rank* evaluation) are still gelatinases.

From a genetic viewpoint in chromosome 11 (11q21-23), 8 genes of human MMPs are *clustered* (MMP-1, -3, -7, -8, -10, -12, -13, -20) (31); and other genes are located on chromosomes 1, 8, 12, 14, 16, 20 and 22.

Matrixins have a primary structure composed of different domains:

A. *N-terminal propeptide* (approximately 80 amino acids) contains a PRCG(V/N)PD preserved sequence. Cysteine contained in this sequence (*cysteine switch*) binds catalytic zinc to maintain the enzyme in its inactive form (pro-MMPs) (5, 36);

B. *Catalytic* (approximately 170 amino acids), includes a HEXXHXXGXXH zinc bond and a protected methionine, thereby forming a structure known as "Met-turn" (6).
The catalytic domain of the MMPs also contains a structural zinc ion and 2-3 calcium ions for stability and enzyme activity. MMPs-2 and -9, for example, receive three fibronectin type II-like repetitions interacting with collagen and gelatine (2, 32);

C. *Peptide*, a signal rich in praline connecting catalytic domain and hemopexin-like domain; an interaction with interstitial collagen on the basis of a molecular model has been theorized (12);

D. *C-terminal hemopexin-like* (approximately 210 amino acids), has an ellipsoid disc form, a fourfold beta-helix plate structure; each plate is composed of four antiparallel beta-sheets and one alfa-helix (19). This domain is necessary during collagenase to process the three-fold helix of interstitial collagen (7). The catalytic domain by itself maintains a proteolytic activity towards the other substrates (11).

MMPs, synthesized as pre/pro-enzymes, are mostly secreted as inactive pro-MMPs and take part in the enzyme degradation of:

a. constituent parts of the extracellular matrix (ECM) (28, 33);

b. basal membrane (BM).

A primary function of MMPs is connected with the daily rhythmic change of collagen structure (interstitial and basal membrane collagen), whose transition modelling dynamics turns out to be essential to:

- maintain and form the cellular environment (during development and morphogenesis);
- cellular migration and angiogenesis (38).

A secondary function concerns the movement of molecules (e.g. IL-1 beta) that as substrates perform some important physiological activities although they are not matrix specific proteins (26, 33).

1 transcription control: which is the main regulation standard of MMPs (16). Production and release can be rapidly induced as needed (28). The inductability of the majority of their genes comes from the action performed for example by:

- cytokines (EGF, TNF-alpha, IL-1 beta, bFGF, PDGF, IL-6);
- hormones and neuropeptides;
- melatonin;
- chemical agents (e.g. phorbol esters, actin stress fiberdisrupting drugs);
- physical and oxidative stress;
- cell oncogenic change;
- B ultraviolet radiations.

Moreover, an increased MMPs gene expression can be under regulated by suppressive factors (e.g. TGF-beta, retinic acid, heparin, glucocorticoids) (23).

2 extracellular activation: the presence of pro-MMPs is the second level of MMPs regulation. The majority of them is secreted by the cell and activated within the matrix by proteases or by non-proteolytic agents (28), low pH and heat treatments (10, 36).

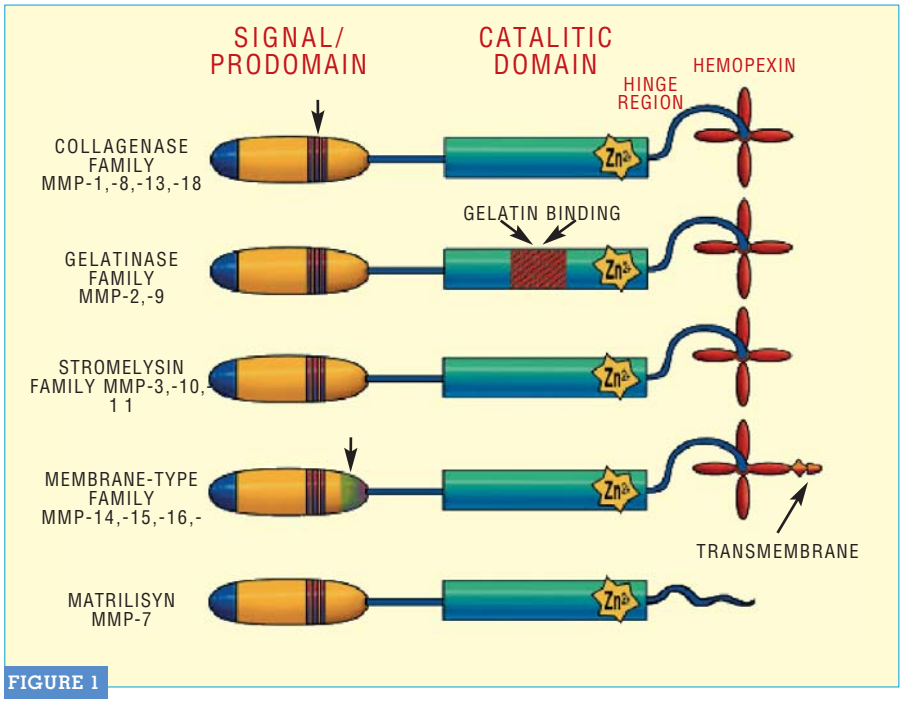


FIGURE 1

Matrix metalloproteases (3, 9, 15, 20).

PHYSIOLOGICAL TISSUE INHIBITORS OF MMPs

The tissue inhibitors of metalloproteases (TIMPs) are a protein family capable of inhibiting the MMPs activity within the extracellular space through bonds non-covalent with their active form (FIGURE 2).

In vertebrata four isoforms of TIMPs have so far been identified to: TIMP-1, TIMP-2, TIMP-3 and TIMP-4 (14, 18).

From the genetic viewpoint, the TIMPs of mammals are included inside intron 5 of the synapsin gene (14). The most important physiological function of TIMPs is connected with the N-terminal domain sequences; the C-terminal domain is involved in interactions with the catalytic domain of some MMPs and the hemopexin domain of MMPs-2 and MMP-9 (8).

The purified and differentiated TIMPs show:

- basic similarity;

- specific structural properties;
- biochemical characteristics and general action guidelines indicating specific functions for each one of them (4).

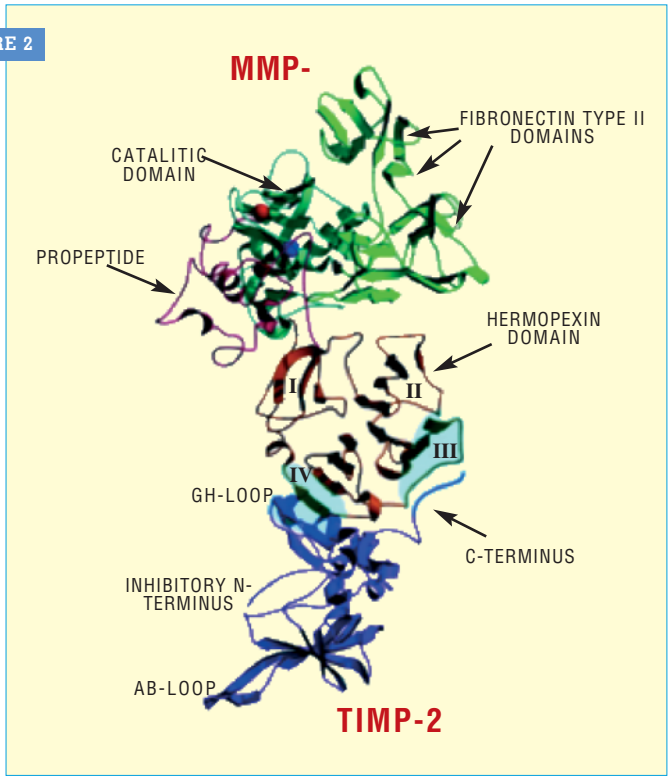
The TIMPs can be associated with membrane proteins, e.g. TIMP-3 (24).

The expression of the matrix TIMPs is carefully regulated during tissue development and remodelling (8); under physiological conditions this expression regulates the metabolic dynamism of the extracellular matrix (13, 18).

The loss of MMPs-TIMPs balancing is connected with changes in the matrix turnover, such as in arthritis, cancer, cardiovascular pathologies, nephritis, neurological disorders, tissue ulceration and fibrosis (28, 38).

FIGURE 2

Structure of the pro-MMP2/TIMP2 complex.



BASE OPERATORS

Within the sphere of interactive complexity hormones, cytokines (TABLE 1), neuropeptides and melatonin (HCNM) give rhythm to the morphofunctional (mfu) capillary vessel-matrix-receptor as Base Operators (BO), i.e., **mediators determining complex chains of events**.

The BO are classified according to radius and mechanism of action. They induce complex reactions connected with molecular specificity and affinity, reversibility of *binding* and synchronization or balancing of matrixins.

The BO move within the matrix in the same way as local chemical mediators do which activate and disappear - very rapidly and by capture - stimulating metabolic changes.

In the Matrix across the information conveyed by HCNM shows:

- expressive property when oriented towards the sender, i.e., the cell receptor (*binding*);
-conative property, when the qualitative and quantitative cipher of the signal changes the receiver's behaviour (up-down regulation);
-phatic property, when the change (metabolè) of the matrix territory maintains and/or discontinues the communication (remodelling).

"The phatic expression" of cytokines has been described in human and rat monocytes through the positive and/or negative modulating action of MMP-9, a metalloprotease involved in the extracellular matrix remodelling (28).

An active role connected with the increase and the expression of MMP-9 has been ascribed to IL-1 beta, TNF-alpha and MIP-1-alpha (39).

IFN-gamma has been identified as a cytokine responsible for MMP-9 inhibition (22, 27).

Basic research confirms the *expressive*, *conative* and *phatic* nature of HCNM - inside the matrix - as a linear extension of an order (tetrametric) which arranges the circadian frame of MMPs-TIMPs from a chrono-biological viewpoint, as well as outlining the capillary vessel-receptor dynamics.

The type of BO, as summarized above, influences considerable matrix changes:

1. 17beta-estradiol stimulates the expression of mRNA, the production of collagen type I and inhibits MMP-1; at a low concentration it could be involved in dermal remodelling and the inhibition of the matrix degradation (29);
2. Interleukin 1-beta plays a key role in cartilage *turnover* by regulating MMPs and TIMPs.

During the culture phase:

- the cell proliferation decreases in the presence of IL-1 beta after 21 days;
- the activity of ALPase significantly decreases after 10 days;
- MMP-1, -2 and -3 considerably increase after 21 days;
- the expression of MMP-13 increases after one day only;
- the expression of TIMP-1 increases after 14 days;
- TIMP-2 decreases after one day, but significantly increases from the third to the fourteenth day.

This data suggests that IL-1 beta stimulates the cartilage turnover, mainly through a MMP-13 increase (1);

3. in patients affected by congestive cardiac disorders, a positive connection has been theorized between noradrenaline and MMP-2, i.e., a potential biochemical link between catecholamines and MMPs;
4. melatonin under regulates the expression and secretion of pro-MMP-9 in a dose-dependent way (in *indomethacin*-induced gastric ulcer) by stopping glutathione depletion and lipid peroxidation in cytosol (17);

5. a positive connection has been suggested between the plasmatic level of noradrenaline and MMP-2 and a negative connection between the level of plasmatic cortisol and MMP-2 itself. This data hints at a further evaluation of those diseases relating to the activation of the hypothalamus-hypophysis-suprarenal glands axis (HPA) and sympathetic adrenomedullary axis (SAM).

To summarize, the BO act as privileged signals that:

- influence the action of the capillary vessel-matrix-receptor morphofunctional unit;
- arrange the molecular language needed for communication between the blood and lymphatic vessel domain and the different cell organizations and between these and other systems;
- move as binding agents in a molecular range which is approximately between 10^{-9}-and 10^{-12} and thereby suitable to receptorial *binding* in terms of affinity;
- induce movement in the MMPs-TIMPs clock (direction of the matrix metabolism) by regulating the continuous remodelling process.

Low concentration and **remodelling** are two important characteristics from the matrix therapeutic modulation point of view.

The hypomole concentration of the medicines acting on HCNM outlines a guideline suitable to the *editing* of vegetative switch-over - according to a metabolic interpretation and a method of access to Physiological Regulation when combined with a chrono-biological remodelling.

The agreement and/or disagreement between **Thyroxin 12X**, **Pyrogenium 6X**, **Cortisonacetat 18X**, **L lacticum Ac 3X** and **HCNM** at a low concentration (molecules moving according to hypomole recurrence), arranges the ante meridian pharmacological use of: IL-6, TNF-alpha, PRL, catecholamines and the post meridian use of IL-10, INF-gamma, melatonin. It also develops a therapeutic approach based on:

- serial wrist calibrated on $t\frac{1}{2}$ of FSH: 3h; IL-2: 2,5 h; cortisol and LH: 1 h;

or

- scanned by a parallel clock; like in the balancing between Th1 (IL-12, INF-gamma, DHEA, PRL), and Th2 (IL-4, cortisol, estradiol, catecholamines).

CONCLUSION

The metabolic regulation clock is capable of making **inconsistency** and **order**, **spontaneity** and **procedures**, **variety** and **unity** coexist. In the perceptive frame of anatomical calling, the matrix time stops as it were interrupted (*cache copy*) in the network of corridors, colonnades, arches and windows of PGs-GAGs.

The *architectural framework* can be refined by the biological information steps.

The photographic vision of the matrix *hyperboloid* outlines the extracellular space as a closed district where the transit and not the dynamism of the biological signal occurs: the monogram slides on glycoproteins like on a waterproof structure.

In an environment in which everything is in constant movement, also the biological information, that is transported by hormones, cytokines, neuropeptides and melatonin, are submitted to the law of adaptation to the present state that is different the subsequent moment.

The flitting of information passes through the matrix area, and thereby frames the discontinuity of the form and its remodelling - suggesting the metabolic *intermittence* as a physiological characteristic of the extracellular space.

The discontinuity creates connections and rebuilding, fills the gaps, and also induces frantic changes that are simultaneously stimulating and precarious.

Even though the matrix is clashing, roundish and pointed, maybe even eccentric, it appears excessive.

The amount of information that crosses the matrix - according to a typical rhythm of matrixins - harmonizes the geometrical network of endothelia with cell receptors… but leads back to another empty space, where the ceiling never arrives, walls are curved, and the glycoprotein brick flooring is diagonal.

The modernity of medical research reveals the extracellular space as a land of dialogue, of continuity between the remodelling of the matrix form and the information syntax… after all… as a place where therapeutic topics, arrangement and time can be drawn from.

And we think of H. H. Reckeweg (30, 34), who rose above the limitations of contemporary thinking of his time by envisioning the grace of a holistic medicine that was not only appealing to modern research, but also gave traditional thought and theory new inspiration and nourishment. ■

References

1. Aida Y., Maeno M., Suzuki N., Shiratsuchi H., Motohashi M., Matsumura H. – The effect of IL-1-beta on the expression of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in human chondrocytes. Life Sci; **2005** Nov 4. 77(25): 3210-21.
2. Allan J.A., Docherty A.J., Barker P.J., Huskisson N.S., Reynolds J.J., Murphy G. – Binding of gelatinases A and B to type-I collagen and other matrix components. Biochem J; **1995** Jul 1. 309(Pt 1): 299-306.
3. Bane C.M., MacCallum R.C., Kiecolt-Glaser J.K., Malarkey W.B., Glaser R. – Stress-related modulation of matrix metalloproteinase expression. J Neuroimmunol; **2002** Dec. 133(1-2): 144-50.
4. Baker A.H., Edwards D.R., Murphy G. – Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J Cell Sci; **2002** Oct 1. 115(Pt 19): 3719-27.
5. Becker J.W., Marcy A.I. et Al. – Stromelysin-1: three-dimensional structure of the inhibited catalytic domain and of the C-truncated proenzyme. Protein Sci; **1995** Oct. 4(10): 1966-76.
6. Bode W., Gomis-Ruth F.X., Stockler W. – Astacins, serralysins, snake venom and matrix metalloproteinases exhibit identical zinc-binding environments (HEXXHXXGXXH and Met-turn) and topologies and should be grouped into a common family, the 'metzincins'. FEBS Lett; **1993**. 331(1-2): 134-40.
7. Bode W. – A helping hand for collagenases: the haemopexin-like domain. Structure; **1995**. 3(6): 527-30.
8. Brew K., Dinakarpandian D., Nagase H. – Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta; **2000** Mar 7. 1477(1-2): 267-83.
9. Briknarova K., Gehrmann M., Banyai L., Tordai H., Patthy L., Llinas M. – Gelatin-binding region of human matrix metalloproteinase-2: solution structure, dynamics, and function of the COL-23 two-domain construct. J Biol Chem; **2001** Jul 20. 276(29): 27613-21.
10. Cha H., Kopetzki E., Huber R., Lanzendorfer M., Brandstetter H. – Structural basis of the adaptive molecular recognition by MMP9. J Mol Biol; **2002** Jul 26. 320(5): 1065-79.
11. Clark I.A., Virelizier J.L., Carswell E.A., Wood P.R. – Possible importance of macrophage-derived mediators in acute malaria. Infect Immun; **1981** Jun. 32(3): 1058-66.

DESCRIPTION	SYNTAX	RESULT
• Defines a series of MMPs-TIMPs connections	• Between the mfu factors and the macrophage system	• Co-ordination of regulating balance • Modelling • Inhibition
TNF-alpha and IL-6 upregulate MMP-9 INF-gamma inhibits MMP-9		

TAB. 1
Base operator
Cytokine.

12. De Souza S.J., Pereira H.M., Jacchieri S., Brentani R.R. – Collagen/collagenase interaction: does the enzyme mimic the conformation of its own substrate? FASEB J; **1996**. 10(8): 927-30.
13. Das S.K., Yano S., Wang J., Edwards D.R., Nagase H., Dey S.K. – Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in the mouse uterus during the peri-implantation period. Dev Genet; **1997**. 21(1): 44-54.
14. Edwards D.R., Beaudry P.P., Laing T.D., Kowal V., Leco K.J., Leco P.A., Lim M.S. – The roles of tissue inhibitors of metalloproteinases in tissue remodelling and cell growth. Int J Obes Relat Metab Disord; **1996**. 20-15.
15. Edwards D.R. – The tissue inhibitors of metalloproteinases: biology and regulation. In Matrix Metalloproteinases Inhibitors in Cancer Therapy. Ed. NJ Clendennin & K Appelt, Totawa; **2000**. 67-84.
16. Fini M.E., Cook J.R., Mohan R. – Proteolytic mechanisms in corneal ulceration and repair. Arch Dermatol Res; **1998**. 290 Suppl: 12-23.
17. Ganguly K., Maity P., Reiter R.J., Swarnakar S. – Effect of melatonin on secreted and induced matrix metalloproteinase-9 and -2 activity during prevention of indomethacin-induced gastric ulcer. J Pineal Res; **2005**. 39(3): 307-315.
18. Gomez D.E., Alonso D.F., Yoshiji H., Thorgeirsson U.P. – Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. Eur J Cell Biol; **1997**. 74(2): 111-122.
19. Gomis-Ruth F.X., Gohlke U., Betz M., Knauper V., Murphy G., Lopez-Otin C., Bode W. – The helping hand of collagenase-3 (MMP-13): 2.7 A crystal structure of its C-terminal haemopexin-like domain. J Mol Biol; **1996**. 264(3): 556-566.
20. Hahn-Dantona E., Ramos-DeSimone N., Sipley J., Nagase H., French D.L., Quigley J.P. – Activation of proMMP-9 by a plasmin/MMP-3 cascade in a tumor cell model. Regulation by tissue inhibitors of metalloproteinases. Ann N Y Acad Sci; **1999**. 878: 372-387.
21. Hijova E. – Matrix metalloproteinases: their biological functions and clinical implications. Bratisl Lek Listy; **2005**. 106(3): 127-132.
22. Johnatty R.N., Taub D.D., Reeder S.P., Turcovski-Corrales S.M., Cottam D.W., Stephenson T.J., Rees R.C. – Cytokine and chemokine regulation of proMMP-9 and TIMP-1 production by human peripheral blood lymphocytes. J Immunol; **1997**. 158(5): 2327-33.
23. Kerrigan J.J., Mansell J.P., Sandy J.R. – Matrix turnover. J Orthod; **2000** Sept. 27(3): 227-33.
24. Leco K.J., Khokha R., Pavloff N., Hawkes S.P., Edwards D.R. – Tissue inhibitor of metalloproteinases-3 (TIMP-3) is an extracellular matrix-associated protein with a distinctive pattern of expression in mouse cells and tissues. J Biol Chem; **1994** Mar 25. 269(12): 9352-60.
25. Lohi J., Wilson C.L., Roby J.D., Parks W.C. – Epilysin, a novel human matrix metalloproteinase (MMP-28) expressed in testis and keratinocytes and in response to injury. J Biol Chem; **2001** Mar 30. 276(13): 10134-44.
26. McCawley L.J., Matrisian L.M. – Matrix metalloproteinases: they're not just for matrix anymore! Curr Opin Cell Biol; **2001**. 13(5): 534-40.
27. Mitola S., Strasly M., Prato M., Ghia P., Bussolino F. – IL-12 regulates an endothelial cell-lymphocyte network: effect on metalloproteinase-9 production. J Immunol; **2003** Oct 1. 171(7): 3725-33.
28. Nagase H., Woessner J.F. Jr. – Matrix metalloproteinases. J Biol Chem; **1999** Jul 30. 274(31): 21491-4.
29. Qu L., Abe M., Yokoyama Y., Ishikawa O. – Effects of 17beta-estradiol on matrix metalloproteinase-1 synthesis by human dermal fibroblasts. Maturitas; **2006** Apr 20. 54(1): 39-46.
30. Reckeweg H.H. – Omotossicologia – Prospettiva per una sintesi della medicina. Guna Ed., Milano; **1988**.
31. Shapiro S.D. – Curr. Opin. Cell Biol. **1998**. 10: 602-608.
32. Steffensen B., Wallon U.M., Overall C.M. – Extracellular matrix binding properties of recombinant fibronectin type II-like modules of human 72-kDa gelatinase/type IV collagenase. High affinity binding to native type I collagen but not native type IV collagen. J Biol Chem; **1995** May 12. 270(19): 11555-66.
33. Sternlicht M.D., Werb Z. – How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol; **2001**. 17: 463-516.
34. Turco L. – *Straight out*, Dottor Reckeweg: oksy´s-mōrós dell'Omotossicologia, ovvero percorsi regolatori in forma di parole. La Med. Biol., **2004**/2. 41-43.
35. Uria J.A., Lopez-Otin C. – Matrilysin-2, a new matrix metalloproteinase expressed in human tumors and showing the minimal domain organization required for secretion, latency, and activity. Cancer Res; **2000** Sep 1. 60(17): 4745-51.
36. Van Wart H.E., Birkedal-Hansen H. – The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc Natl Acad Sci USA; **1990** Jul. 87(14): 5578-82.
37. Visse R., Nagase H. – Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure,function, and biochemistry. Circ Res; **2003** May 2. 92(8): 827-39.
38. Woessner J.F. Jr. – Role of matrix proteases in processing enamel proteins. Connect Tissue Res; **1998**. 39(1-3): 69-73.
39. Zhang Y., McCluskey K., Fujii K., Wahl L.M. – Regional and differential expression of gelatinases in rat brain after systemic kainic acid or bicuculline administration. Eur J Neurosci; **1998**. 10(11): 3358-68.

Figg. 1 and 2 have been revised from a graphic viewpoint and taken from the web sites as follows:
- www.emdbiosciences.com
- www.theses.ulaval.ca/2004/21981/21981001.png

Author's address

Dr. Lucio Turco
– Specialist in Psychiatry at P.-H. in Formia
Via Appia Lato Napoli, 1
I – 04023 Formia (LT)