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Meeting Report

Cellular differentiation and death in a renaissance castle

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The VII International Conference on Transglutaminases and Protein Crosslinking Reactions: Ferrara, Italy, 14–17, September 2002.

Transglutaminases (TGs) comprise a family of multifunctional enzymes that have important roles in assembly of covalently crosslinked structures during cell differentiation and apoptosis. These enzymes catalyze covalent modification of proteins including amine incorporation, formation of protein–protein crosslinks, site-specific deamidation, and isopeptide bond cleavage. Several forms are known, each with a different tissue distribution and mechanism of regulation. Selected enzymes in this family also possess G protein activity and functioning in cell signaling.

The VII International Conference on TGs and Protein Crosslinking Reactions convened from September 14 to 17 in the Estense Castle in Ferrara, Italy. The meeting considered the basic structural features of TGs, the functional role of these proteins and production and identification of their reaction products. This meeting was especially timely in light of the increasing importance of these enzymes in physiology and pathology. Dr Carlo Bergamini and colleagues at the University of Ferrara organized the meeting with the assistance of an International Scientific Board, including Richard L Eckert (Cleveland), Laszlo Fesus (Debrecen), Martin Griffin (Nottingham), Gerry Melino (Rome) and Donatella Serafini-Fracassini (Bologna). The conference was attended by over 150 scientists from Italy and other European (Czech Republic, Finland, France, Germany, Holland, Hungary, Spain, Sweden and UK) and overseas (Australia, Canada, China, Israel, Japan, Korea, New Zealand, Thailand, and USA) countries.

The meeting opened with an overview by Professor Peter Davies of the University of Texas Medical School on the history of TG research, and the prospects for future advances in the postgenomic, that is, proteomic era. While recent developments in the field were discussed during the scientific sessions, the historic perspective was emphasized during the ceremony of award of an Honorary Degree in Medicine and Surgery from the University of Ferrara to Professor Laszlo Lorand, of the Northwestern University School of Medicine (Chicago).

Professor Lorand is a discoverer of TGs, and, as Professor Lorand himself detailed during his Lectio Doctoralis, his seminal research established an understanding of the important role that one these enzymes, factor XIII, has in blood clotting.

Many topics were presented; reflecting the breath of functions now assigned to the TG proteins. There are now at least nine mammalian TG proteins. These include types 1–7, coagulation factor XIII, which all have TG catalytic activity, and band 4.2, an erythrocyte membrane protein, devoid of catalytic activity but highly homologous in structure to other members of the TG family. These enzymes are expressed in a tissue-, differentiation- and disease-specific manner. Factor XIII, and type 1 (TG1) and type 2 (TG2) are the best-studied TG enzymes. Factor XIII is an important enzyme in the blood-clotting cascade and in wound repair, and is an important risk factor in severe cardiovascular diseases. TG2 is expressed in a wide range of tissues and is involved in cell death and survival. TG1 is present in many surface epithelia anchored to the plasma membrane and functions to catalyze covalent isopeptide bond formation during terminal differentiation of keratinocytes. The activity of all these enzymes to catalyze protein crosslink formation requires the presence of high concentrations of calcium as an essential activator.

TG structure and function

Several talks addressed the functional role of TGs and particularly TG2. TG2 enzyme encodes four domains, including the amino-terminal domain that binds fibronectin and integrin; the catalytic core domain that binds calcium contains the nuclear localization motifs and catalyzes isopeptide bond formation; the β-barrel 1 region that participates in the organization of the GTPase site and contributes residues

involved in binding guanine nucleotides; and the β-barrel 2 region that interacts with PLCδ. TG2 can localize in the cytoplasm or nucleus, and with membranes or organelles. It also associates with the extracellular matrix through a poorly understood externalization mechanism. The cellular environment likely influences the activity of this enzyme. For example, the low free calcium levels and high guanine nucleotide triphosphate levels characteristic of the cytosol favor the activity of TG2 resembling that of classic G proteins (i.e. signal transduction), while the high intracellular calcium levels typical of dying cells favor the protein–protein crosslinking activity. Studies described by SC Park and colleagues suggest that GTP is required to stabilize the transamidation activity of TG2, and additional studies by Y Saito (Tokyo) suggest that TG2 may possess a protein disulfide isomerase (PDI) activity, which is not inhibited by GTP and does not require calcium. Dr E Candi from Professor Melino's laboratory (Rome) presented work suggesting that TG5, which is structurally similar to TG2, also is functionally similar. A presentation by P Steinert (Bethesda) described a model whereby the two substrates that are ultimately linked by an isopeptide bond are positioned in a common tunnel that forms at the TG3 active site. These studies highlight the important role of the local environment on activity of the TG enzymes.

Nonmammalian TGs and biotechnology

An interesting session focused on the TG enzymes derived from plants (maize, tropical flowers, etc.), fruit flies, worms, slime mold, and bacteria with presentations from L Fesus (Debrecen), M Griffin (Nottingham), PLR Bonner (Nottingham), D Serafini-Fracassini (Bologna), K Hitomi (Nagoya), A Worratao (Nakon Ratchasima, Thailand), and others.

Many of these enzymes display homology with the mammalian TGs. In plants, TGs function in assembly/organization of the cell wall, and are involved in root development, pollen tube growth, and cell death.

A membrane-associated TG is present in the tonoplast membranes of *Pisum sativum*, and light-activated TG forms may reside in chloroplasts of some species. A TG in *Physarum polycephalum* (slime mold) is homologous to the mammalian type II form, includes a GTP-binding site, is localized at the cell surface, and appears to be involved in signal transduction in response to injury.

A remarkable feature regarding these enzymes is the conservation of function and sequence motifs compared to the mammalian forms. In addition, it was reported that the *Caenorhabditis elegans* (filarial worm) genome encodes a specific TG, with two PDI active sites; this protein, expressed in the endoplasmic reticulum, catalyzes disulfide bond formation, isomerization, and reduction. This enzyme is not homologous to the TGs present in other species. However, this PDI also catalyzes protein isopeptide bond formation both *in vitro* and *in vivo*, and thus appears to possess transglutaminase activity. This is interesting, as under certain conditions, some mammalian TG isoforms also function as PDIs. Fish TG, from the tropical tilapia, was also described as an 85 kDa protein that possesses classical TG activity.

A separate session focused on the biotechnological applications of TG. Owing to its protein–protein crosslinking function, TG is considered to have important commercial applications. Presentations at the meeting indicate that progress is being made using various TGs to modify textiles and food products, and to immobilize proteins on insoluble supports. It can be expected that these enzymes will reach commercial scale utilization in the future.

TG substrates

L Fesus (Debrecen) presented a comprehensive overview of known TG substrates, which include endogenous proteins involved in intermediary metabolism, proteins of the cytoskeleton, and proteins of the extracellular matrix. M Kaartinen and M McKee (Montreal) described α_2HS-glycoprotein, bone sialoprotein, and osteopontin as novel extracellular matrix TG substrates, and suggested a role for TG in facilitating bone and tooth adhesion. The general role of TGs as modifiers of the extracellular matrix was particularly evident as outlined by M Griffin (Nottingham). It is also clear that TG-dependent modification also has a role in modulating signal transduction processes. For example, in studies from the K Aktories Laboratory (Freiburg), the TG-dependent transglutamidation of Rho at GLN_{136} was shown to promote Rho dissociation from membranes and termination of Rho-dependent signaling. In another example, by R Eckert and colleagues (Cleveland), it was suggested that signal transduction by the calcium-responsive S100 proteins is terminated by TG-dependent S100 polymerization. In addition, as discussed by C Esposito (Naples) and other speakers, dietary proteins, notably gliadins from cereals, are TG substrates, and their covalent modification by tissue TG appears to be an essential step in the pathogenesis of celiac disease. As additional substrates are identified, it is becoming increasingly clear that a comprehensive knowledge of substrate targets will be required to understand the context-dependent role of TG. To track the identification of new target, a proposal was launched for construction of a TG Substrate Data Bank.

TG-associated diseases

The role of TGs in the pathogenesis, as well as treatment of disease, was an additional important focus of the meeting. Celiac disease is characterized by atrophy of villi in the jejunum and the appearance of a flattened mucosa in response to dietary glutens exposure.

This is caused by TG2 that deamidates glutens to produce immunoreactive gluten peptides. These peptides trigger an immune response that destroys the jejunal lining. A secondary immune response produces anti-TG2 autoantibodies against TG2 that are diagnostic of the disease. That these antibodies can modify the catalytic activity of TGs and thereby influence the local production of cytokines was reported by S Auricchio (Naples) and by Maki's Lab (Tampere).

Some celiac disease patients also display epidermal manifestations, which are manifest in a disease called dermatitis herpetiformis. The molecular basis for the skin response is not understood, but results presented by

M Sardy (Budapest) suggest that these changes may be caused by a gliadin-induced autoimmune reaction against TG3, a TG isoform present in the granular layer of human epidermis.

Huntington's disease is a neurodegenerative disease caused by CAG triplet expansion in the gene encoding huntingtin (htt) that leads to a production of an htt protein containing an elongated stretch of polyglutamine residues. Ubiquinated htt tends to aggregate in the nucleus of neurons leading to their death. It has been suggested that this aggregation may involve TG-dependent crosslinking at these glutamine residues. An interesting study showed that overexpression of htt in TG2 knockout mice results in htt aggregation, suggesting that TG2 is not necessary for aggregate formation. However, in spite of aggregate formation, the htt-positive/TG2-negative mice had improved brain cell survival, improved motor performance and survived better than htt-postive/TG2-positive mice. This finding suggests that TG2 has an important role in this disease, but that it is not required for htt aggregate formation. In addition, B Festoff (Kansas City) described evidence supporting a role for TG in the pathogenesis of vascular-dependent neuropathologies, such as ischemic and traumatic diseases, including post-traumatic Parkinson's disease.

TG2 also has an important role in normal tissue remodeling. For example, B Graham (Sidney) reported impaired healing of skin in TG2 knockout mice. Study of fibroblasts derived from these mice suggests that the cells are biochemically prepared to move to close the wound (high G protein function, disassembled stress fibers, etc.), but do not, apparently because of impaired adhesion, spreading, proliferation, and migration. D Aeschlimann (Cardiff) presented additional studies showing that TG2-negative cells are unable to repopulate a monolayer scratch, suggesting that TG2 has an important role in migration. A joint study by D Aeschlimann (Cardiff), G Melino (Rome), M Piacentini (Rome), and L Fesus (Debrecen) indicated that cellular apoptosis could be induced in the liver and thymus of TG2 knockout mice. However, although the cells are dead, they are not efficiently removed from the tissue by the macrophages. This suggests that the macrophages from TG2-negative animals are functionally deficient. TG also has a role in renal scarring. The tissue damage associated with renal scarring is associated with increased TG2 expression, increased TG2 release from renal tubule cells and increased deposition of isopeptide bonds. TS Johnson and colleagues (Sheffield) described studies showing that TG2 levels are markedly increased under conditions of low pH, hypoxia, and high glucose. The authors suggest that treatments aimed at controlling acidosis, hypoxia, and glucose levels are likely to reduce the incidence of renal scarring.

TG2 has an interesting role in cancer biology. In general, expression of activated TG2 in tumor cells results in cell death, and TG2 overexpression may be therapeutic for cancer treatment. M Griffin (Nottingham) described studies showing that intratumor injections of TG2 in mice reduced tumor size and increased animal survival.

Retinoids were reported to enhance apoptosis in liver cells and this increase was associated with high TG2 expression. Likewise, melanoma cell migration was inhibited by theophylline treatment and this was related to increased TG2 levels (S Lentini, S Beninati, Rome). Professor K Mehta (Houston) presented the data suggesting that the cell signaling function of TG2 may provide a growth/survival advantage to breast cancer cells expressing high levels of TG2 via its G protein activity, and that the crosslinking activity of the enzyme is not active in highly transformed cells. Thus, by preventing intracellular calcium levels from rising high enough to activate TG, these tumor cells may escape cell death.

A Belkin (Rockville) discussed the regulation of TG2 expression showing that TG2 expression requires ERK activity. M Piacentini presented a discussion suggesting that cellular stress may be the major factor responsible for the induction of TG activity and that in many cases this induction may be an effort by the cell at self-protection.

The role of factor XIII in cardiovascular disease and clotting disorders was also discussed. T Noll and G Wozniak (Giessen) described the ability of factor XIII to reduce the incidence of myocardial edema during heart surgery in children. However, if this self-protective effort is carried to extreme, the cells undergo apoptosis. As reported by L Muszbek (Debrecen), the expression of variant forms of factor XIII, particularly the $Val_{34}Leu$ variant, is related to a decreased risk of cardiovascular disease, further underlying the importance of this protein in the control of microcirculation, and differentiation in hematopoiesis (A Ichinose, Yamagata). P Bishop (Seattle) and GD Argenio (Naples) reported that restoration of factor XIII levels, using recombinant factor XIII, stops colitis in a rat model of the disease. Both Japanese and German groups reported production of knockout mice for factor XIII (XIII) last year at the ICTH meetings in Paris. In discussion, A Ichinose mentioned that XIIIA knockout mice have a tendency to bleed, which can be corrected by administration of factor XIII. In contrast, XIIIB knockout mice do not show any phenotype. Further investigation will be needed to clarify the functional roles of Factor XIII.

Final comments

This interesting meeting highlighted the explosive increase in knowledge regarding the function of TGs, and elucidation of their essential role in regulating cell differentiation, cell migration, cell proliferation, tissue remodeling, wound healing, cell and tissue adhesion, programmed cell death, blood clotting and development. Attention was also focused on the recognition that disordered expression and/or activity of these enzymes is an important underlying cause in many diseases, including Huntington's disease, renal fibrosis, celiac disease, and Alzheimer's disease. Interesting new research is now beginning to explore the role of TG in other chronic human diseases, including arthrosis and diabetes mellitus. On the whole, these studies suggest that we are now close to being able to transfer knowledge generated from this basic research to the bedside. Finally, the identification and characterization of new TG forms in plants, fruit flies, worms, etc. suggest new ways of using these enzymes for modification of commercial processes and products. It will be interesting in the coming years to observe the development of the TG field.

Acknowledgments

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