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Transglutaminases

What Links Celiac Disease, Thrombosis, Skin and Hair?

transglutaminases

The class of enzymes known as transglutaminases is of increasing interest to biomedical scientists. This interest is documented in several excellent scientific reviews published in recent years. Here we would like to invite you on a short trip through applied science, diagnostics and drug development in this remarkable and burgeoning field of transglutaminase research.

Dr. Martin Hils and Dr. Ralf Pasternack are cofounders of Zedira. Zedira is a venture capital-backed company located in Darmstadt, Germany. Zedira develops and commercializes products for transglutaminase R&D and for diagnostic purposes. In addition, the company develops small molecule blockers targeting transglutaminase.

Background

Excellent science is the fundamental cornerstone of the life science industry. Science requires a great deal of sweat, time and money. It takes a large amount of patience and investment to transform scientific knowledge into commercially viable products. Our story starts 50 years ago when the term transglutaminase (TG) was first introduced into the scientific literature. Today, we know that we are not talking about a single enzyme, but about a family of eight different active isoenzymes in the human body. Table 1 provides a concise overview of the members of the transglutaminase family and their functions.

For some of the human transglutaminases the physiological functions still remain unclear. But they have one feature in common – Transglutaminases are "nature's biological glue".

What does that mean?

As an illustration, consider that transglutaminases provide the barrier function to our skin and bring shape and curls to our hair. The three isoenzymes involved in these processes are named epidermal transglutaminase (TG1), keratinocyte transglutaminase (TG3) and transglutaminase 5. Specifically, these isoenzymes cross-link hair and skin proteins via a unique mechanism. Transglutaminases catalyze the transfer of a glutamine side chain of a protein substrate to a protein-bound lysine, resulting in the formation of a very stable inter- or intra-protein bond, the so called isopeptide bond. Typically, insoluble high molecular weight protein polymers are generated.

The physiological role of plasma Factor XIII is quite similar. In the final step of the blood coagulation cascade the pro-enzyme (i.e. Factor XIII) is activated by thrombin to its active form (i.e. Factor XIIIa). The activated Factor XIIIa cross-links fibrin leading to the macroscopic and life-saving fibrin clot we all know. Looking a little more deeply, it is not only the simple cross-linking reaction of fibrin that is of fundamental importance to our survival, but also the transglutaminase-catalyzed attachment of functional proteins to the clot. The main protein that should be mentioned in this regard is α2-antiplasmin. Decorating the clot with α2-antiplasmin provides resistance against fibrinolysis by plasmin.

Perhaps the most enigmatic enzyme in the transglutaminase family is tissue transglutaminase (TG2). Tissue transglutaminase is ubiquitously expressed throughout the human body. In addition to the above-mentioned cross-linking activity, TG2 acts as a G-protein and is therefore involved in signal transduction. Much evidence suggests that tissue transglutaminase expression and activation is important in apoptosis (programmed cell death) and in inflammatory events. When externalized via an as yet unknown mechanism, the enzyme contributes to cell adhesion and to modulation of the extracellular matrix.

From a medical point of view, celiac disease is a fascinating topic involving extracellular tissue transglutaminase. This chronic inflammatory disease affects the small intestine and is due to intolerance toward the protein gliadin in the gluten fraction of wheat and related cereals. Celiac disease affects about 1% of the population, justifying the enormous efforts that have been directed toward elucidating the pathophysiological and molecular mechanisms involved. Basically, it was known for many years that, in addition to gluten intolerance, patients with celiac disease had a genetic predisposition. However, this was not the whole story and a third piece of the puzzle was sought for many years. The missing piece was eventually found to be tissue transglutaminase. Surprisingly, celiac disease is an autoimmune disease and tissue transglutaminase is the autoantigen.

Transglutaminases in Diagnostics

The identification, about ten years ago, of tissue transglutaminase as the autoantigen in celiac disease opened the way for major improvements in the diagnosis of this common disease. Recent research has suggested that some other autoimmune diseases can also result from the production of autoantibodies directed against specific transglutaminases (Table 2). Some patients with celiac disease exhibit ataxia (gluten ataxia). Recently, antibodies directed against neuronal transglutaminase (transglutaminase 6) have been identified in patients with gluten ataxia. Since prolonged damage of the central nervous system is largely irreversible, screening of celiac patients for antibodies directed against neuronal transglutaminase should be seriously considered.

The above discussion serves to emphasize the fact that transglutaminase research has currently generated a double digit annual million Euro market for diagnostics in Europe and North America. We anticipate an explosive increase in the market in the near future.

Transglutaminase-Based Drugs

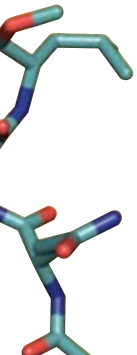
Transglutaminases are already available via the pharmaceutical market. For example, Coagulation Factor XIII purified from human plasma is used for the prevention and treatment of bleeding complications and abnormalities in blood clotting associated with Factor XIII deficiencies. However, since only a few hundred people worldwide suffer from Factor XIII deficiency, other medical applications, such as its use as a possible drug for patients undergoing cardiac surgery, must be addressed to expand the market potential. This is underlined by recombinant human Factor XIII, which is currently being tested in advanced clinical trials.

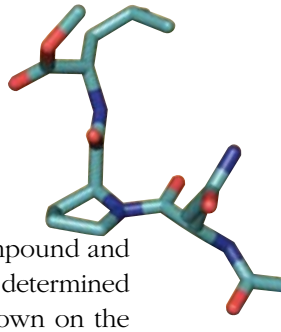
Other Human Transglutaminase Deficiencies

Lamellar Ichthyosis, also a rare disease, is caused by transglutaminase 1 deficiency. The severe symptoms associated with this disease emphasize the crucial role

Table 1. The Transglutaminase Family

Name of the enzyme	Synonyms	Function
Factor XIII	Plasma Transglutaminase; fibrin stabilizing factor	Blood coagulation and wound healing
Transglutaminase 1 (TG1)	Keratinocyte Transglutaminase	Cornified envelope formation, skin differentiation
Transglutaminase 2 (TG2)	Tissue Transglutaminase	Apoptosis and cell differentiation; signal transduction; extracellular matrix stabilization; cell adhesion
Transglutaminase 3 (TG3)	Epidermal Transglutaminase	Cornified envelope formation, skin differentiation
Transglutaminase 4 (TG4)	Prostate Transglutaminase	Semen coagulation (in rodents)
Transglutaminase 5 (TG5)		Cornified envelope formation, skin differentiation
Transglutaminase 6 (TG6)	Neuronal Transglutaminase	Unknown
Transglutaminase 7 (TG7)		Unknown





of transglutaminase 1 in skin maturation. The patients suffer from impaired synthesis of the cornified envelope, leading to a high transepidermal water loss and a reduced barrier function of the skin.

Acral peeling skin syndrome is caused by transglutaminase 5 deficiency. The disease is characterized by shedding of the outer epidermis of the dorsa of feet and hands.

Since recombinant transglutaminase 1 as well as recombinant transglutaminase 5 are now available, topical administration of these agents should be considered as a therapeutic option.

Current Approaches in Transglutaminase-Related Drug Development

In addition to the suggested strategy mentioned above of supplementing transglutaminases where the disease is clearly due to a transglutaminase deficiency, there is a strong medical need for blocking the activity of these enzymes by specific inhibitors when transglutaminase activity is detrimental. In celiac disease, intrinsic tissue transglutaminase activity is the key player in the game. Due to the low pH-value in inflammatory intestinal tissue, transglutaminase catalyzes not only cross-linking reactions but also a hydrolytic reaction. Tissue transglutaminase deamidates glutamine residues in cereal protein (gluten/gliadin) leading to a highly immunogenic product. Production of autoantibodies against tissue transglutaminase is a consequence. The molecular mechanisms in celiac disease suggest a promising therapeutic approach. Since the crucial event in the chronic disease state is the interaction between transglutaminase and gliaden in the alimentary tract, blocking of the extracellular tissue transglutaminase activity in the lower intestine may break the vicious circle leading to inflammation and erosion of intestinal villi.

Accordingly, efficient and specific inhibitors for tissue transglutaminase are promising new therapeutic entities. In our laboratories we are developing small-molecule compounds to block tissue transglutaminase activity by using rational drug design methods. The structure of the complex formed between our lead compound and human tissue transglutaminase has been determined using high resolution crystallography as shown on the cover. This has clearly accelerated the design of new peptidomimetic compounds.

Moreover excessive/aberrant transglutaminase activity has been suggested to contribute to a number of other diseases. Examples include the chronic inflammatory states associated with hepatic fibrosis and cirrhosis, as well as with renal and lung fibrosis. Recent publications indicate the involvement of transglutaminase in cancer progression. When considering cross-linking and insoluble protein deposits, neurodegenerative diseases come to mind. It is important to note that insoluble protein deposits are hallmarks of Huntington disease as well as senile dementia of the Alzheimer type and many other neurodegenerative diseases. However, it remains to be seen whether inhibiting brain transglutaminases (including transglutaminase 2 and transglutaminase 6) will significantly delay the progression of neurotoxic events. A straight- forward approach will be to test new compounds in suitable animal models of neurodegenerative diseases.

Last, but not least, in addition to tissue transglutaminase, Coagulation Factor XIII is also considered to be a suitable target in patients at risk for thrombotic events. Specific blockers are predicted to help save lives by a rather limited intervention in the blood coagulation pathway.

We are convinced that new insights into the (patho)physiology of transglutaminases will lead to novel diagnostic and therapeutic approaches in the near future.

We hope you enjoyed the journey from transglutaminase-based science to market.

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Table 2. Autoimmune Disorders Linked to Transglutaminases

Antigen	Symptoms	Disease
Transglutaminase 2; Tissue Transglutaminase	Inflammation of the small intestine	Celiac Disease (Coeliac Disease)
Transglutaminase 3; Epidermal Transglutaminase	Itchy deposits of transglutaminase 3 and IgA in the skin	Dermatitis Herpetiformis, Morbus Duhring
Transglutaminase 6; Neuronal Transglutaminase	Ataxia/Neuropathy due to autoantibodies affecting the nervous system	Gluten ataxia, gluten sensitive neuropathy