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Regulation of hematopoiesis in the freshwater crayfish, *Pacifastacus leniusculus*

role of transglutaminase

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Abstract

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The freshwater crayfish, *Pacifastacus leniusculus*, has been used as a model for studying hematopoiesis or blood cell production or hematopoiesis and immunity. The work of this thesis aims to investigate the impact of factors such as ROS signaling, Ast1, and the PVF/PVR signaling pathway in controlling stem cell behavior during hematopoiesis and specifically the role of the crosslinking enzyme transglutaminase (TGase) in regulation of hematopoiesis.

The role of ROS in crayfish hematopoiesis was characterized by using the antioxidant named NAC to inhibit ROS production. Low ROS level resulted in a prolonged decrease in hemocyte numbers and a combined injection of LPS and NAC caused a slower rate of new hemocyte production. A low ROS level in cell cultures supplemented with crude Ast1 was found to inhibit cell spreading and a high extracellular TGase activity was detected on the surfaces of APC and HPT cells. We suggest that ROS serves as a prime signal to control proliferation and differentiation of progenitor cells by affecting extracellular TGase activity. We reported an inhibitory effect of Ast1 on TGase enzyme activity and on its crosslinking activity and consequently Ast1 affects the clot formation and thus coagulation by inhibiting the crosslinking activity of the TGase enzyme. Secretion of the clot protein (CP) and the production of CP filament network between spreading cells were observed in HPT cell cultures *in vitro*. In the presence of CP together with Ast1 in 3D-collagen-I cultures, HPT cells were found to be more elongated and they formed chains of cells throughout the surrounding matrix. In the HPT tissue, CP was located around the HPT cells or around the lobules of HPT, and thus, CP was demonstrated to be a part of ECM and to possibly function together with collagen in generating a suitable environment for HPT progenitor cells. The inhibition of PVF/PVR downstream signaling pathway by Sunitinib malate resulted in a dramatic change of cell morphology and induction of an increase cell surface area during cell culture. The addition of crude Ast1 into the cell cultures *in vitro* enhanced this effect. Consequently, cell migration was stimulated and a high extracellular TGase activity on HPT cell surface was found after this inhibition. In conclusion, the work in this thesis provides new insight in understanding the role of the extracellular matrix (ECM) and extracellular TGase activity in controlling stem cell activity.

Keywords: Ast1, clotting protein, crayfish, hematopoiesis, PVF/PVR, ROS, transglutaminase

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To my Mom

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Junkunlo K**, Söderhäll I, Söderhäll K, Noonin C. (2016). Reactive oxygen species (ROS) affect transglutaminase activity and regulate hematopoiesis in a crustacean. *J Biol Chem*. 291 (34):17593-17601.

II Sirikharin R, **Junkunlo K**, Söderhäll K, Söderhäll I. (2017). Role of astakine1 in regulating transglutaminase activity. *Dev. Comp. Immunol*. 76:77-82.

III **Junkunlo K**, Söderhäll K, Noonin C, Söderhäll I. PDGF/VEGF related receptor affects transglutaminase activity to control cell migration during crustacean hematopoietic. *Stem Cells and Development* (In Press).

IV **Junkunlo K**, Söderhäll K, Söderhäll I. Clotting protein – an extracellular matrix (ECM) protein involved in crustacean hematopoiesis. (Submitted)

Additional Publications

Udompetcharaporn A, **Junkunlo K**, Senapin S, Roytrakul S, Flegel TW, Sritunyalucksana K. (2014). Identification and characterization of a QM protein as a possible peptidoglycan recognition protein (PGRP) from the giant tiger shrimp *Penaeus monodon*. Dev. Comp. Immunol. 46(2):146-154.

Saelee N, Noonin C, Nupan B, **Junkunlo K**, Phongdara A, Lin X, Söderhäll K, Söderhäll I. (2013). β-thymosins and hemocyte homeostasis in a crustacean. PLoS One. 8(4):e60974.

Junkunlo K, Prachumwat A, Tangprasittipap A, Senapin S, Borwornpinyo S, Flegel TW, Sritunyalucksana K.A. (2012). A novel lectin domain-containing protein (LvCTLD) associated with response of the white leg shrimp *Penaeus (Litopenaeus) vannamei* to yellow head virus (YHV). Dev. Comp. Immunol. 37(3-4):334-341.

Contents

Abbreviations

Adgf-A	Adenosine deaminase growth factor-A
Antp	HOX factor Antennapedia
APC	Anterior proliferation center
Ast	Astakine
BM	Bone marrow
BMP	Bone morphogenetic protein
CC	Crystal cells
CHF	Crustacean hematopoietic factor
c-Met	Hepatocyte growth factor receptor (HGFR)
Col	EBF transcription factor Collier
CP	Clotting protein
CZ	Cortical zone
ECM	Extracellular matrix
FGF	Fibroblast growth factor
GC	Granular cell
HC	Hyaline cell
HGF	Hepatocyte growth factor
Hml	Hemolectin
HPT	Hematopoietic tissue
HSC	Hematopoietic stem cell
HUVEC	Human umbilical vein endothelial cell
Ig	Immunoglobulin domain
KPI	Kazal proteinase inhibitor
LC	Lamellocytes
LPS	Lipopolysaccharide
LvTG I	Litopenaeus vannamei transglutaminase type I
LvTG II	Litopenaeus vannamei transglutaminase type II
MBL	Mannose binding lectin
mTOR	A mammalian target of rapamycin inhibition
MZ	Medullary zone
NAC	N-acetylcysteine
NADPH oxidase	Nicotinamide adenine dinucleotide phosphate-oxidase
NHDF	Normal human dermal fibroblast cell
NK	Natural killer cell
PC	Plasmatocytes

PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
PK	Prokineticin
ProPO	Prophenoloxidase
PSC	Posterior signaling center
PVF	PDGF- and VEGF-related factor
PVR	PDGF- and VEGF-related receptor
Rpr	Reaper (proapoptotic gene)
RNAi	RNA interference
ROS	Reactive oxygen species
SGC	Semigranular cell
SMC	Smooth muscle cell
SOD	Superoxide dismutase
Src	A tyrosine kinases family protein
STG I	*Penaeus monodon* transglutaminase type I
STG I	*Penaeus monodon* transglutaminase type II
TEM	Transmission electron microscope
TGase	Transglutaminase
TGF-β	Transforming growth factor beta
TK	Tyrosine kinase
Trol	Heparin sulfate proteoglycan Terribly Reduced Optic Lobes
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
vWF-D	Von Willebrand factor type D domain

Introduction

The freshwater crayfish, *Pacifastacus leniusculus,* is a model for studying invertebrate immunity and also blood cell production (Cerenius and Söderhäll, 2017; Söderhäll, 2016). Invertebrate immune system mainly relies on innate immune responses, and the blood cells or hemocytes are cells that circulate in the hemolymph and play important roles in this defense. The functions of crayfish hemocytes are similar with cells of the myeloid lineage in vertebrates. Myeloid cells include monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, dendritic cells, and megakaryocytes or platelets. In vertebrates, during injury or infection cells of the myeloid lineage rapidly migrate into infected tissues, and act by phagocytosis or by secretion of inflammatory cytokines (Arango Duque and Descoteaux, 2014). Like the myeloid cells in vertebrates, hemocytes in invertebrates function in removing and killing microbes by phagocytosis or encapsulation. Hemocytes also have ability to secrete a variety of antimicrobial peptide (AMP) to mediate microbial elimination (Diamond et al., 2009; Jiravanichpaisal et al., 2006). In addition, in both invertebrates and vertebrates the coagulation or blood clotting is known as a first barrier in preventing entering and spreading of microbes and leaking of hemolymph. Clot formation is based on a combination of coagulation factors and cell-derived factors, for example transglutaminase (TGase) enzyme and the clotting protein (CP) in crustaceans, which was first discovered and described in freshwater crayfish (Kopácek et al., 1993; Hall et al., 1999; Theopold et al., 2004). Therefore, due to their decisive importance for animal host defense, studies of the blood cell production or hematopoiesis are important to provide more understanding about the innate immunity of crustaceans and invertebrates in general and crayfish in particular.

Hematopoiesis

Hematopoiesis is a complex process by which new blood cells develop and are released from hematopoietic tissues into the circulation. Regulation of hematopoiesis, especially by specific transcription factors is evolutionary conserved from invertebrates to vertebrates (Söderhäll, 2016). The hematopoietic process is tightly regulated in order to balance the proliferation and differentiation of hematopoietic stem cells (HSC). The homeostasis of cells

is controlled by the coordination of internal factors for example transcriptional regulators and external factors such as a signaling molecules from surrounding tissues (Gattazzo et al., 2014). The hematopoietic regulation has been well characterized in various model organisms such as in mouse, zebrafish, and *Drosophila* (Crozatier and Vincent, 2011; Jagannathan-Bogdan and Zon, 2013). However, very few studies have been carried out in other species. For this reason, molecular studies of hematopoiesis in different invertebrates are needed to fill the gap in the present knowledge. In addition to *Drosophila,* the freshwater crayfish also serves as a good model for studies of hematopoiesis. There are two main reasons why freshwater crayfish, is a good model for studies of the molecular regulation of hematopoiesis. First, this kind of crayfish has a long lifespan like many vertebrates. They can live up to at least 20 years and their hematopoiesis continues throughout their lifetime. Second, the hematopoietic tissue (HPT) is easy to remove from the animals, and the HPT can be cultured *in vitro* as a whole tissue or as isolated cells, which enable studies of different factors and their effect on proliferation and differentiation. This capacity of HPT cells to proliferate and differentiate *in vitro* provides a good opportunity to study the regulation of hematopoiesis in detail.

In humans, fetal hematopoiesis first takes place in the yolk sac and then relocates to the fetal lever. Finally, adult hematopoiesis is translocated to the bone marrow (BM). HSC then resides in the BM and hematopoiesis occurs throughout lifetime in humans (Wasnik et al., 2012). HSC maturation can be divided into different stages such as proliferation, commitment or fate determination and differentiation (Seita and Weissman, 2010). HSCs, which reside in the BM have a unique ability to replicate and differentiate into all different mature blood cell types of the lymphoid and myeloid lineage. After maturation and release of mature cells into circulation, the survival times of the blood cells are limited. The red blood cells continue to live about 120 days, while the life times for white blood cells vary between an hour to a few days, and for platelets is around 10 days (Alberts et al., 2002). Thus, a constant replacement of new blood cells is required to substitute the old cells. However, during certain conditions such as during an infection or injury more blood cell production is stimulated. Thus, when needed, HSCs in the BM are activated and start to replicate and further differentiate into specific cell types.

In insects and crustaceans, hematopoiesis occurs in a specific tissue and the maturation stages of HSC can also be divided into different stages as in human. After maturation, the mature hemocytes are present in the hemolymph during embryonic as well as during adult stages. The lifespan of circulating hemocytes varies from one day to several weeks (Grigorian and Hartenstein, 2013). Furthermore, approximately 10% of the hemocyte population is pro-

duced daily to replace old hemocytes (Grigorian and Hartenstein, 2013). Similar to vertebrates, rapid restoration of hemocytes happens during specific conditions. A drop in the number of circulating hemocytes occurs immediately after injection of lipopolysaccharides (LPS) or injection of β-1,3-glucans (fungal cell wall components) in crustaceans, followed by a rapid recovery (Persson et al., 1987; Smith et al., 2015). Thus, the blood cell or hemocyte homeostasis is essential for survival of the animal.

Crayfish hemocytes

In crayfish and other invertebrates, hemocytes are key players in innate immunity both in humoral and cell-mediated immune responses. For humoral responses, hemocytes produce and secrete AMPs and the prophenoloxidase (proPO) system components to combat pathogens. In cell-mediated responses, hemocytes kill invaders by phagocytosis, encapsulation, and cytotoxic reactions (Cerenius and Söderhäll, 2017). In crustaceans particularly shrimp, crab, and crayfish, hemocytes are divided into different types based on morphology, granule content and function. Three types of hemocytes are found, namely the hyaline cell (HC), semi-granular cell (SGC) and granular cell (GC) (Johansson et al., 2000). The most abundant hemocyte in crayfish hemolymph is the SGC (Figure 1), which contains small granules and is involved in early recognition, coagulation and encapsulation (Lin and Söderhäll, 2011). The GC (Figure 1) has large granules, which contains the proPO system components and different AMPs (Johansson and Söderhäll, 1985; Sricharoen et al., 2005). HC is small and contains very few or no granules. In the hemolymph of healthy intermolt *P. leniusculus*, the most abundant cell types in circulation are SGCs and GCs, whereas HCs are rarely found. A similar situation was observed in shrimp and lobster (Smith et al., 2016). HC has been proposed as an immature stage of the SGC and GC lineages, which are released from HPT during an infection and then develop into mature cells (Söderhäll, 2016). In contrast to crayfish and shrimp, HC is an abundant cell type in crabs and they appear to be responsible for phagocytosis (Jia et al., 2016; Söderhäll and Smith, 1983). In *Drosophila melanogaster*, hematopoiesis generates three different hemocyte types; plasmatocytes (PC), crystal cells (CC) and lamellocytes (LC). PC is the dominating hemocyte type in *Drosophila*. In addition, PC can be transformed into LC during parasite infestation (Stofanko et al., 2010). PC is a non-granular cell, which has phagocytic capacity and can be compared to the SGC in crayfish (Söderhäll, 2016). LC is also a non-granular cell but larger than PC and functions in encapsulation. The CC contains granules containing the proPO activating systems like GC in crayfish (Khadilkar et al., 2017; Smith et al., 2016). In *Drosophila* and other insects, prohemocytes are located in the so-called lymph gland, which is equivalent to the crustacean HPT (Grigorian

and Hartenstein, 2013). The very last step of hemocyte maturation occurs when hemocytes express their mature hemocyte markers. This step possibly occurs after the cells are released from the HPT into the circulation since the mature hemocyte markers, such as proPO are hardly detected in the HPT (Söderhäll et al., 2003).

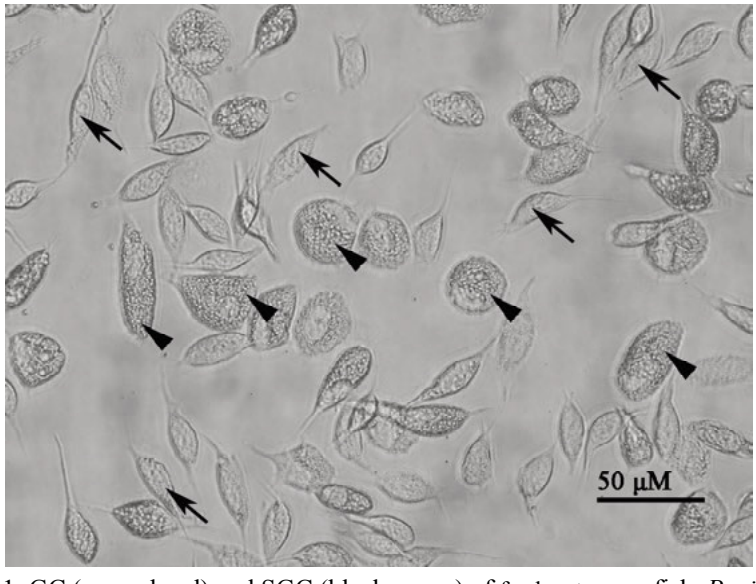

Figure 1. GC (arrow head) and SGC (black arrow) of freshwater crayfish, *Pacifastacus leniusculus*.

Crayfish hematopoietic tissue (HPT)

Among crustaceans, the hematopoietic tissue has been characterized in most detail in crayfish (see references in Söderhäll, 2016). Crayfish HPT is a separate organ, which covers the stomach close to the brain (Figure 2). In black tiger shrimp (*Penaeus monodon*), HPT is located in different areas of the cephalothorax mainly at the dorsal side of the stomach. In shrimp, the HPT expands to the maxilipeds and the antennal gland (Van de Braak et al., 2002). In Chinese mitten crab (*Eriocheir sinensis*), the HPT is located underneath the carapace (Jia et al., 2016). In oyster (*Crassostrea gigas*), stem cell-like cells were discovered in the gill epithelium and in hemolymph, which may support the hypothesis that gill epithelium serves as a HPT in bivalves (Jemaà et al., 2014).

The HPT in crayfish is a thin sheet, which consists of loosely or densely packed lobules with cells surrounded by connective tissue (Figure 2). Inside the lobules are hematopoietic precursors; undifferentiated/proliferative cells, and immature

cells (prohemocytes and maturing hemocytes) (Chaga et.al.1995). In addition, extracellular molecules, which are secreted by cells inside the HPT, are present, that not only function as a supportive structure for progenitor cells but also are involved in regulating cell behavior (Paper IV). The extracellular matrix (ECM) functions in hematopoietic regulation by controlling self-renewal and differentiation/proliferation of HSC. In shrimp, the HPT lobules are surrounded by a fibrous connective tissue and are partly embedded in muscle tissue (Van de Braak et al., 2002). In crayfish, collagen type I and IV were found to be major components of the ECM in HPT (Junkunlo et al., 2016; Söderhäll, 2013). Crayfish HPT can be divided into three different parts; posterior HPT, anterior HPT and the anterior proliferation center (APC) (Figure 2A). APC is a small area in the middle of anterior HPT, and it is located in proximity to the brain (Noonin et al., 2012). In crayfish HPT, different stages of progenitor cells are found inside lobules and some of them are located between lobules (Figure 2B) (Chaga et al., 1995). The characterization of hematopoietic progenitor cells in HPT by using transmission electron microscope (TEM) revealed that at least 5 morphological cell types are present in crayfish HPT (Chaga et.al.1995). Type I progenitor cells have a large nucleus, which are surrounded by a very thin layer of cytoplasm. Furthermore, type I cells are usually closely attached to the ECM. Type II is a proliferating cell, but more differentiated than type I cells. The proliferating cells in APC are mostly similar to type I and type II cells. About 75% of the mitotic cells in HPT tissue are type II cells. These cells are located close to type I cells. Type III cells are more differentiated than type I and type II cells. Type IV cells contain more electron dense granules that are found in type III cells, granules which are similar to the ones found in GCs. Consequently, type III and IV cells are suggested to be GC precursors. The morphology of type V cells is different from the other cell types, and it is suggested that type V is a SGC precursor. In conclusion, these 5 types of progenitor cells differentiate into two hemocyte lineages; SGC, and GC (Chaga et al., 1995; Lin and Söderhäll, 2011; Söderhäll, 2016). In addition, the progenitor cells located in the APC divide more rapidly compared to cells in the posterior HPT (Lin et al., 2010; Noonin et al., 2012). In *in vitro* cultures, the APC cells form spherical clusters, whereas HPT cells form monolayers (Noonin et al., 2012).

The arrangement of cells in crayfish HPT is different from that in the *Drosophila* lymph gland. The lymph gland in the fly consists of many lobes alongside the dorsal vessel, and the different progenitor cells are clearly distributed in specific areas of the lymph gland (Jung et al., 2005). This arrangement is different from the crayfish HPT, in which the progenitor cells of different stages are not precisely placed in specific areas. However, some areas have more proliferating cells and other areas have more differentiated cells (Noonin et al., 2012; Söderhäll, 2016). In *Drosophila* lymph gland, the primary lobes are most important for hemocyte development and can be separated into three different zones; the cortical zone (CZ) where maturing hemocytes are found; and the

medullary zone (MZ) where immature hemocytes are located. The primary lobes also include the posterior signaling center (PSC) where a unique population of cells is located. The cells in the PSC area are not identified as prohemocytes or mature hemocytes but signals from the PSC are required for blood cell progenitor maintenance (Grigorian and Hartenstein, 2013). A parasite infestation stimulates an increase in ROS production in the PSC and in the MZ, which results in induction of LC production (Owusu-Ansah and Banerjee, 2009). Laminarin injections in crayfish provide a similar effect as in *Drosophila* PSC, and a high ROS level was observed in the APC prior to new hemocyte release (Noonin et al., 2012). LPS or laminarin injection cause a rapid loss in circulating hemocyte number and then a rapid recruitment of new hemocytes from HPT is initiated. The increase in ROS production in APC after LPS or laminarin injection is probably due to high metabolic activity of the APC cells during the increased proliferation and differentiation (Noonin et al., 2012). Perhaps the APC can act like the PSC in *Drosophila* as a signaling center in sending signals or be a source of differentiating molecules for regulation of stem cell activity in crayfish (Junkunlo et al., 2016; Noonin et al., 2012). The APC and its regulatory function may also be involved in stress responses and in the environmental control of hemocyte homeostasis. Moreover, a recent report shows that APC has a direct connection to the crayfish neurogenic niche in the brain (Chaves da Silva et al., 2013). Interestingly, hemocyte precursors were shown not only to be involved in hematopoiesis but also to provide neural stem cells for adult neurogenesis (Benton et al., 2014).

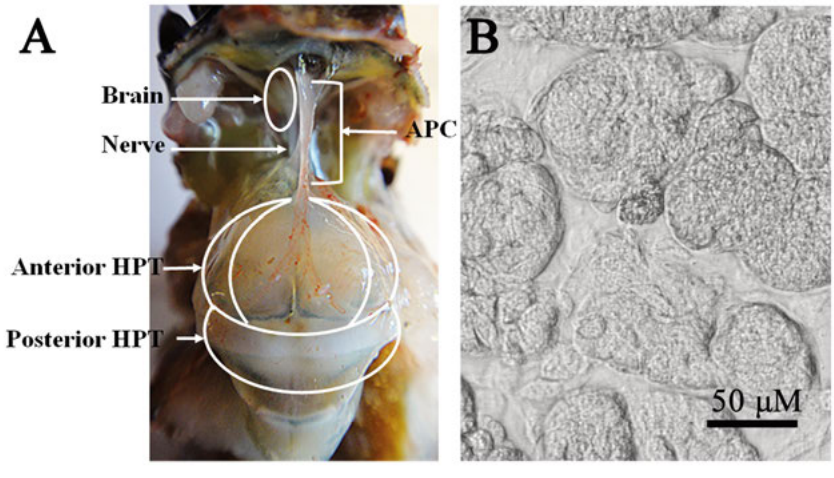

Figure 2. The hematopoietic tissue (HPT) in *P. leniusculus*. A) The HPT is a thin sheet located at the dorsal part of the stomach and the Anterior Proliferation Center (APC) links HPT and brain. B) Detail of a whole mount HPT under microscope, HPT consists of lobules containing hematopoietic progenitor cells.

Extracellular matrix (ECM) and niche

Although, HSCs in general are self-renewing through cell-autonomous processes, HSCs activity also requires extensive interaction with the surrounding microenvironment or niche (Anthony and Link, 2014). A stem cell niche is a special microenvironment that consists of HSCs and other cell populations such as mesenchymal cells that regulate HSC self-renewal, differentiation, and proliferation. A niche is a regulatory unit that limits the entry of HSCs into the cell cycle. Therefore, HSCs are in a quiescent stage and are protected from unnecessary proliferation or from errors in DNA replication (Boulais and Frenette, 2015). Inside a niche, the HSCs are controlled through cell-cell interactions and by secretory molecules such as growth factors. Also interactions between progenitor cells with ECM proteins occur during stem cell fate regulation (Anthony and Link, 2014). Thus, the ECM is an essential component in the stem cell niche that both directly and indirectly modulates the stem cell behavior. As a supportive structure, the ECM is commonly found lining under the epithelial layer or surrounds a connective tissue. The ECM is composed of a mixture of molecules, which vary in composition and concentration, both within and between tissues, leading to different ECM properties (Baker and Chen, 2012). Usually, an ECM consists of two main classes of extracellular macromolecules, which are glycosaminoglycans (GAGs) and fibrous proteins, which are secreted locally and assemble into a crosslinked insoluble matrix. GAGs are usually linked to proteins by covalent bonds forming proteoglycans. Fibrous proteins include collagen, elastin, fibronectin, and laminin (Alberts et al., 2002). The basement membrane is mainly composed of laminins and collagen type IV (non-fibrillar), whereas the connective tissue is organized by collagen type I and II (fibrillar) (Mouw et al., 2014). Besides the ECM structural proteins, other regulatory factors for example cytokines, growth factors, and enzymes are integrated as parts of the ECM. Several ECM crosslinking enzymes such as TGase, lysyl oxidase and prolyl hydroxylase, stabilize and conjugate the ECM molecules. Moreover, growth factors for example TGF-β, BMPs, PDGFs, Wnts, and Hedgehogs and also some cytokines can bind to ECM proteins and activate the downstream signaling pathways to regulate cell behavior. Other ECM-associated proteins such as mucins, C-type lectins, syndecans, and matrilins have been considered as important molecules to form a niche for specific cell types (Naba et al., 2012).

In mammals the hematopoietic niche is a complex structure composed of several cell types, including osteoblasts, perivascular cells, endothelial, Schwann cell, sympathetic neuronal cells and macrophages (Morrison and Scadden, 2014). The hematopoietic niche in insects and crustaceans is probably a complex system as well. In *Drosophila*, PSC is suggested to function as a hematopoietic niche that provides signaling molecules required for

maintenance of blood cell progenitors in the lymph gland (Pennetier et al., 2012). PSC development depends on the EBF transcription factor Collier (Col) and the HOX factor Antennapedia (Antp). In a Col mutant, the PSC is abnormal and the larval lymph glands exhibit a massive decrease in blood cell progenitors and increased hemocyte differentiation (Benmimoun et al., 2015). In Antp mutant larvae there is no PSC, and instead CC differentiation increases (Mandal et al., 2007). However, a recent, study showed that PSC did not function as a niche in *Drosophila* hematopoiesis, but that the signals from the PSC might directly or indirectly function in progenitor cell fate determination (Benmimoun et al., 2015). The introduction of a genetic mutation by expressing the pro-apoptotic gene named reaper (Rpr) in specific tissues, provides an opportunity to kill specific cells in the PSC. Interestingly, in flies with a PSC-less lymph gland this had no effect on progenitor cell maintenance and did not cause an increase of blood cell differentiation (Benmimoun et al., 2015). Furthermore, PSC is located within the primary lobe of lymph gland, which is far from 2nd and 3rd lymph gland lobes. How PSC controls the progenitor cell differentiation in the 2nd and 3rd lobe still needs to be investigated. For this reason, PSC was suggested as a signaling center to modulate signals for progenitor cell maintenance and differentiation instead of being a niche (Benmimoun et al., 2015). In crayfish, the APC was suggested to act as a source of signals in controlling differentiation of progenitor cells, mainly due to presence of short ROS pulses in this area (Noonin et al. 2012).

The heparin sulfate proteoglycan Trol (Terribly Reduced Optic Lobes) is the *D. melanogaster* homolog of the vertebrate protein perlecan. Trol is expressed as part of the ECM found in the lymph gland (Grigorian et al., 2013). Furthermore, Trol is formed as a basement lamellae and surrounds the densely packed prohemocytes. Loss of Trol causes a dramatic change in the ECM conformation and results in a smaller lymph gland and also induces premature hemocyte differentiation (Grigorian et al., 2013). In vertebrates, perlecan is present both as a basement membrane and on the cell membrane surface (Yurchenco, 2011). Perlecan functions in morphogenesis of the skeleton (Melrose et al., 2006) and during angiogenesis (Jiang and Couchman, 2003). In addition, perlecan is found to interact with a variety of growth factors including fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) on chondrocytes, smooth muscle cells (SMC), and endothelial cells (Dreyfuss et al., 2009). The interaction of perlecan and growth factors induces cellular proliferation, differentiation and ECM production (Lord et al., 2014). Like in mammals and *Drosophila*, crayfish APC and HPT are composed of connective tissue together with ECM. Collagen type I and IV are major components of this ECM in both the APC and the HPT (Lin et al., 2008). However, before the studies presented in this thesis, functional studies of ECM in crusta-

cean hematopoiesis are limited, and mainly include studies on cuticle synthesis and remodeling during molting or studies of the cuticle that covers the digestive system (Glazer et al., 2010; Glazer and Sagi, 2012). Apart from studies of the crosslinking enzyme TGase for ECM stability, we report a potential role of the clotting protein (CP) as a part of ECM in crayfish HPT (paper IV).

Clotting protein

Blood clotting in both vertebrates and invertebrates is a part of the immune response. Clot formation is the first line of defense preventing the entering and spreading of pathogens, once there is a wound in the cuticle (Cerenius and Söderhäll, 2013; Hall et al., 1999). In vertebrates, blood coagulation occurs as a result of a proteolytic enzyme cascade, finally forming an insoluble clot network. The vertebrate clotting or coagulation pathway is a series of reactions in which inactive enzyme precursor serine proteinases and their co-factors are activated to become active enzymes. Then, the active enzymes catalyze the next reaction in the cascade. At the last step of reaction, factor XIIIa, which belong to the TGase enzyme family creates intermolecular ε-(γ-glutamyl) lysine bonds between fibrin molecules to stabilize the clot formation (for a review see Smith et al., 2015). In invertebrates, the coagulation mechanisms of the freshwater crayfish, horseshoe crab and shrimp have been characterized in molecular detail (Kopácek et al., 1993; Theopold et al., 2004; Yeh et al., 1999). The clotting reaction in horseshoe crab involves a proteolytic cascade that in the final step activates the clotting enzyme, which is similar to the coagulation process in vertebrates, but probably not so complicated. Coagulogen is the final clotting protein in horseshoe crab hemolymph and may functionally be compared with fibrinogen in vertebrates, however the size and sequence are completely different (Cerenius et al., 2010; Osaki and Kawabata, 2004). Likewise, the proteinases of the horseshoe crab clotting cascade have no similarity with their invertebrate counterparts (Cerenius et.al 2010). The clotting cascade in horseshoe crab is stimulated by microbial components such as LPS, finally resulting in the conversion of a soluble protein (coagulogen) into an insoluble aggregate (coagulin) (Kawabata, 2010). In crayfish and shrimp, coagulation depends on TGase activity in crosslinking of a specific plasma clotting protein without a proteolytic cascade. Clot formation was first deciphered in crayfish and is induced when TGase is released from hemocytes or other tissues and is activated by calcium ions in plasma (Hall et al., 1999; Kopácek et al., 1993). In horseshoe crab a TGase has been characterized, but this enzyme does not recognize coagulin as a substrate in the clotting reaction, but acts later to stabilize the clot (Osaki and Kawabata, 2004). In *Drosophila,* PCs secrete a clotting protein named Hemolectin (Hml), which is a major component of the clot matrix. Then, TGase, a homologue for clotting factor XIIIa mediates the cross-

link of Hml and other proteins (fondue, gelsolin and apolipophorin II etc.) to form a clot network (Gábor et al., 2017; Theopold et al., 2014).

Crustacean CPs was first purified, cloned and characterized from hemolymph of freshwater crayfish (Hall et al., 1999; Kopácek et al., 1993) and later characterized in several other crustaceans (for references see Maningas et al., 2013). Unlike horseshoe crab coagulogen, which is located in hemocytes, crayfish and shrimp CP are plasma proteins. Crayfish CP is a homodimeric glycoprotein of two identical subunits linked together by disulfide bonds. Each 210-kDa subunit contains free lysine and glutamine sidechains and can be covalently crosslinked with other homodimers by TGase (Hall et.al.1999; Kopácek et.al. 1993 and Wang et.al 2001). The CP sequence was first identified from a hepatopancreas cDNA library and shown to be similar to vitellogenins (Hall et.al 1999). Furthermore, crayfish CP contains the D domain of mammalian von Willebrand factor (vWF-D). Crustacean CP does not share sequence similarity with fibrinogen in vertebrates or coagulogen in horseshoe crabs. The vWF-D is a large multimeric glycoprotein involved in mammalian blood coagulation, and the D domain is important for clot polymerization (Jorieux et al., 2000). The importance of crayfish CP as a substrate for TGase in the clotting reaction was further studied by electron microscopy. In the presence of TGase and Ca^{2+}, CP molecules were crosslinked into long and flexible branched chains (Hall et al., 1999). In crayfish plasma TGase can be released from hemocytes or HPT into the hemolymph. Then, Ca^{2+} activates the clotting reaction and CP molecules present in plasma are crosslinked into large aggregates (Hall et al., 1999). In a crayfish transcriptome analysis (BioProject ID: PRJNA259594), high expression of CP mRNA was detected in HPT libraries. In addition, the expression of crayfish CP mRNA was found in most tissues but very low in hemocytes. Similarly, in shrimp, CP mRNA was hardly expressed in hemocytes (Cheng et al., 2008; Yeh et al., 1999). In paper IV, the function of CP in crayfish HPT was further investigated in more detail (see Paper IV below).

Crayfish Hematopoietic Factors

Astakines (Ast)

Cytokines are small molecules, which are secreted by immune cells and play important roles in innate immune responses and inflammatory responses (Beck, 2014). Moreover, cytokines are involved in growth and differentiation of various immune cells such as macrophages, mast cells, B-cells, T-cells, and Natural Killer (NK) cells (Arango Duque and Descoteaux, 2014). In invertebrates, cytokines are secreted by a variety of cell types and act as

important coordinators of immune responses. In crustaceans, a new group of cytokines named Astakines (Ast) was first discovered in *P. leniusculus* and *P. monodon* (Söderhäll et al., 2005). Ast is related to vertebrate prokineticins (PK), but lack the conserved vertebrate N-terminal AVIT-sequence (Söderhäll et al., 2005). PKs have been reported to regulate the development of the nervous systems, and to be involved in immunity, reproduction and hematopoiesis in mammals (LeCouter et al., 2004; Miele et al., 2010). Two isoforms of Ast have been identified in crayfish (Lin et al., 2010). The first astakine1 (Ast1) was isolated from crayfish plasma with a property to induce HPT stem cell proliferation in HPT cell cultures as well as under *in vivo* conditions (Söderhäll et al., 2005). Later, another astakine (Ast2) was characterized, and it may have a role in GC differentiation, although this is much less clear (Lin et al., 2010).

Ast1 is mainly expressed in hemocytes and HPT. This protein has a direct function in balancing differentiation/proliferation of hematopoietic progenitor cells and is essential in controlling the release of new hemocytes into the circulatory system. Injection of native (purified) or a recombinant Ast1 can induce a rapid increase of hemocyte production (Söderhäll et al., 2005). Ast1 induces proliferation and differentiation of HPT progenitor cells into the SGC lineage (Lin and Söderhäll, 2011), while Ast2 appears to be involved in increasing the number of mature GCs (Lin et al., 2010). In addition to crayfish, astakines have been found in other invertebrates such as shrimp, spiders and insects (Söderhäll, 2016). In black tiger shrimp (*P. monodon*), Ast was shown to stimulate hemocyte proliferation in the hematopoietic tissue (Hsiao and Song, 2010). Crayfish Ast1 lacks the N-terminal AVIT amino acids, which is present in vertebrate PK. The N-terminal AVIT region is important for binding to its G-protein coupled receptors in vertebrates (Bullock et al., 2004). According to this difference, the receptor of crayfish Ast1 might not be the same as for vertebrates PK. In a previous report crayfish Ast1 was found to bind to a β-subunit of F_1ATP synthase on the cell surface of actively proliferating cells in HPT (Lin et al., 2009). An Ast homologue from white shrimp (*Litopenaeus vannamei*) was also reported to bind to the β-subunit of ATP synthase and was involved in preventing viral infection (Liang et al., 2015). In addition, shrimp crustin Pm4 and transglutaminase I (STG I) was reported as RNA binding proteins that down-regulate Ast protein expression at the post-transcriptional level (Chang et al., 2013). In crayfish, we have shown that Ast1 affects the ECM structure by regulating extracellular TGase activity. The addition of crude Ast1 into HPT cultures caused a decrease in extracellular TGase activity (Lin et al., 2008). Moreover, Ast1 directly regulated TGase enzymatic activity by acting as a non-competitive inhibitor, and inhibited the gel-forming reaction between CP and TGase (paper II: Sirikharin et al., 2017). The importance of Ast1 and TGase in reg-

ulating the structure of the ECM was further investigated in a 3D collagen-I culture (see Paper IV below).

PDGF/VEGF-related receptor (PVR)

Members of the PDGF/VEGF family have been implicated in cell proliferation, cell differentiation, cell migration, vascular development, angiogenesis, and neural development (Andrae et al., 2008; Chen et al., 2013). Recent studies about PDGF/VEGF related proteins in invertebrates are mainly performed in the model organism *Drosophila,* and information about this family of growth factors in aquatic invertebrates is missing so far.

PDGFs and their receptors (PDGFRs) have been used as a model for functional studies of the growth factor and receptor tyrosine kinases. Mammalian PDGFs are categorized into class I and II by the presence of basic retention motifs or CUB domains (Andrae et al., 2008). PDGF is a dimeric glycoprotein that consists of two subunits that form a dimer of disulfide-linked polypeptide chains (Heldin et al., 1979). The protein domain structure of PDGFRs consists of five extracellular immunoglobulin (Ig) domains and an intracellular tyrosine kinase (TK) domain (Yarden et al., 1986). The binding between PDGFs and their receptors on cell surface membranes are essential for the transduction of extracellular signals into cells. The downstream signaling is mediated by a series of intracellular signaling cascades through its association with Src homology 2 (SH2) domain-containing adaptor proteins (Chen et al., 2013). Based on protein domain organization and functional characterization, *C. elegans* and *Drosophila* PDGF/VEGF-related protein ligands (PVFs) are most similar to VEGF (Holmes and Zachary, 2005; Kipryushina et al., 2015). Three different isoforms of PVF (PVF1-3) and a single PDGF/VEGF-related protein receptor (PVR) have been reported in *Drosophila* (Parsons and Foley, 2013). *Drosophila* PVF/PVR signaling is involved in hemocyte migration during embryonic development (Parsons and Foley, 2013) and hemocyte homeostasis in adult flies (Mondal et al., 2014). Recently, a PDGF/VEGF related factor named EsPVF1 was studied in the Chinese mitten crab, *Eriocheir sinensis*. The EsPVF1 contains a conserved PDGF/VEGF domain and a CXCXC motif as in other PDGF/VEGF family proteins. An infection or an injury induces up-regulation of EsPVF1 and the resulting release of norepinephrine and dopamine. This result may indicate that EsPVF1 is involved in the regulation of the immune and neuroendocrine systems of this crab (Li et al., 2013), but certainly more detailed information is necessary before such a link can be made. From crayfish transcriptome analysis (BioProject ID: PRJNA259594), four different partial sequences, which show sequence similarity to the PDGF/VEGF family of proteins, were found. The putative receptor protein Pl_PVR1, GenBank accession number KY444650, could be used as a mature hemocyte marker, since Pl_PVR1 is

mainly expressed in hemocytes in similarity to *Drosophila* PVR. In contrast to *Drosophila* PVR, Pl_PVR1 contains a nonspecific kinase domain (STYKc), which can phosphorylate the OH group of serine, threonine or tyrosine amino acid residues, whereas *Drosophila* PVR contains a specific tyrosine kinase domain (TyrKc). *Drosophila* PVR is used as a cell differentiation marker since PVR positive cells are located mainly in the CZ. Moreover, *Drosophila* PVF1 is produced in the PSC, and the PVF/PVR signaling acts as an equilibrium signal, which promotes progenitor cell maintenance or differentiation via the adenosine deaminase-related growth factor A (Adgf-A) pathway. The high expression of crayfish Pl_PVR1 in mature hemocytes as opposed to immature HPT cells may suggest that Pl_PVR1 functions as a differentiation signal similar to PVR in *Drosophila*. In mammals, PDGF is known as a key regulator, which directly induces cell migration during embryogenic development and during wound healing (Schneider et al., 2010). Similarly, *Drosophila* PVF2 and PVF3 are reported as chemotactic factors in the regulation of hemocyte migration during development and wound healing in the same manner as in mammals (Wood et al., 2006). Recent studies in mammals also show that tissue TGase at the cell surface, functions both as a transamidating enzyme and as an extracellular scaffold protein in the ECM in controlling cell migration (Wang and Griffin, 2012). Furthermore, the binding of cell surface tissue TGase with PDGFR and integrin effectively enhanced the PDGF/PDGFR downstream signaling which promoted cell migration and proliferation (Zemskov et al., 2012). In crayfish, the function of PVF/PVR downstream signaling pathway and its role in stem cell activity is shown and discussed below (see details in Paper III).

Transglutaminase (TGase)

TGase belongs to a large family of intracellular and extracellular enzymes. The activity of this enzyme is Ca^{2+}-dependent (Eckert et al., 2014). TGases play a role in a variety of essential processes, including blood coagulation, skin formation, and signal transduction. TGase generates bonds between lysine and glutamine residues to form ε-(γ-glutamyl) lysine bonds (Shibata and Kawabata, 2015). In mammals, nine TGase enzymes have been identified and eight of them have active enzyme properties (Eckert et al., 2014). TGase can function both intra- and extracellularly. The first TGase called a tissue TGase, was discovered in 1959 from guinea pig liver (Eckert et al., 2014). Tissue TGase is a multifunctional protein, which has an important role in vascular biology. Tissue TGase is expressed in various tissues both as intracellular and extracellular forms (Nurminskaya and Belkin, 2012). This enzyme is involved in cell-matrix interactions, cell attachment and cell population expansion. Loss of tissue TGase on cell membranes of endothelial cells causes a reduction in cell number. This is a result of the increase of cell cycle arrest, loss of cell adhesion and apoptosis induction (Nadalutti et al.,

2011). In invertebrates, TGase has been identified in many species for example crayfish, horseshoe crab, grasshopper, starfish and fruit fly (Shibata and Kawabata, 2015; Sugino et al., 2002). Invertebrate TGases are involved in innate immunity especially clot formation, which was first described in crayfish (Wang et al., 2001). The sequence of invertebrate TGases show similarity with mammalian Plasma Factor XIIIa (Wang et al., 2001). Factor XIIIa or the plasma TGase is an enzyme in the blood coagulation system that stabilizes the fibrin clot. Two different isoform of TGase was reported in black tiger shrimp (*P. monodon)*. These were assigned as STG I, and STG II. STG I is mainly expressed in the hematopoietic tissue and is involved in hemocyte proliferation (Chen et al., 2005; Huang et al., 2004). STG II is characterized as a hemocyte TGase and is involved in coagulation (Chen et al., 2005). Two different types of TGase were also found in the Pacific white shrimp (*L. vannamei)* and were named as LvTG I, and LvTG II (Chang et al., 2016). LvTG II is more closely related to STG II than to STG I. LvTG II functions as a clotting enzyme involved in hemolymph coagulation (Yeh et al., 2013). Both LvTG I and LvTG II show an effect in response to *Vibrio alginolyticus* infection and induce the proliferation of circulating hemocytes (Chen et al., 2014). In the starfish, TGase has a role in ECM remodeling during embryonic development (Sugino et al., 2002).

In crayfish, TGase, (GenBank accession no. AF336805) was first isolated from a hemocyte cDNA library (Wang et al., 2001). TGase is an abundant protein in both HPT, and hemocytes, especially in SGCs (Lin et al., 2008). The promoter region of the crayfish TGase gene consists of GATA binding motifs, which are also present in the regulatory region of the human factor XIIIa gene (Kida et al., 1999; Lin et al., 2008). GATA transcription factors are DNA-binding proteins, which play an important role in hematopoietic processes in mammals as well as in invertebrates (Chlon and Crispino, 2012). The finding of GATA binding domains in the crayfish TGase promoter indicates a role of TGase in hematopoiesis like in mammals (Lin et al., 2008). In mammals, a tissue TGase links various substrate proteins such as ECM proteins like fibrinogen, fibronectin or laminin on the cell surface by enzymatic as well as by non-enzymatic mechanisms (Nurminskaya and Belkin, 2012). Furthermore, a tissue TGase affects cell behavior by regulating the ECM stability and composition, which leads to inhibition of angiogenesis and tumor growth (Jones et al., 2005). In human umbilical vein endothelial (HUVEC) cell cultures, inhibition of a tissue TGase results in inhibition of fibronectin deposition together with interfering the matrix-bound VEGFA/VEGFR signaling pathway (Wang et al., 2013).

Co-localization of extracellular TGase with ECM components was detected in crayfish HPT both *in vivo* and *in vitro* (Paper I and Paper IV). For this reason, extracellular TGase might be involved in hematopoiesis by promot-

ing cell-ECM interactions. In addition, extracellular TGase activity was shown to be much higher in less differentiated cells residing in the crayfish HPT than in cells migrating out of the tissue (Lin et al., 2008). A decrease in mRNA expression of TGase by RNAi resulted in spreading of HPT cells. When Ast1 was added into the culture medium, similar cell spreading behavior as after TGase knockdown was observed and cells started to migrate (Lin et al., 2008). Ast1 has an impact on extracellular TGase activity, but not on the TGase mRNA expression (Lin et al., 2008). BrdU incorporation assays together with TGase activity detection showed that crayfish TGase activity is high in proliferating cells (Lin et al., 2008). Moreover, our recent studies showed that the PVF/PVR signaling pathway is involved in regulating extracellular TGase activity and in controlling cell migration (see details in Paper III).

Reactive oxygen species (ROS)

HSCs in mammals are found in the bone marrow (BM) and the hematopoietic processes occur in this area. In BM, HSCs are kept in a quiescent, non-motile state by interacting with stromal cells and osteoblasts, forming specialized niches (Yu and Scadden, 2016). Quiescent, proliferating, and differentiating stem cells have different metabolic rates (Zhang and Sadek, 2014). The specialized environment inside the BM is important for maintaining the HSCs in a quiescent state with low proliferation rates. High ROS level produced by excessive proliferation or from uncontrolled cell-cycle entry of progenitor cells can damage and impair stem cell function (Eliasson and Jönsson, 2010). The niche and its components do not only function as supportive structures for progenitor cells, but also protect stem cells from stress, such as accumulation of ROS and from DNA damage. However, ROS can also serve as internal signals, which are required for progenitor cell differentiation (Zhang and Sadek, 2014). High level of ROS induces the differentiation of HSCs into the myeloid lineage both in mammals and *Drosophila* (Sinenko et al., 2012; Vincent and Crozatier, 2010). In *Drosophila,* high ROS levels were found in hematopoietic progenitor cells in the MZ and in the PSC (Sinenko et al., 2012). In addition, higher ROS level in the PSC occurred after a wasp infestation and led to an increased number of LCs (Sinenko et al., 2012). In crayfish, high ROS level was observed in the APC where actively proliferating cells are located. LPS injection in crayfish results in an increased ROS level in the APC, which is followed by a gradual increase of circulating hemocytes (Noonin et al., 2012). Moreover, low level of ROS was required to regulate the extracellular TGase activity to maintain progenitor cells inside of the crayfish HPT (paper I: Junkunlo et al., 2016). In vertebrates, the levels of ROS in the hematopoietic niche are regulated by intrinsic factors, for example nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) activity or extrinsic factors such as cytokines and

growth factors. Stress and inflammatory responses cause high ROS levels, which further induce stem cell differentiation and enhance cell motility (Ludin et al., 2014). The cytokine hepatocyte growth factor (HGF) and its receptor c-Met was reported to regulate ROS production in BM. c-Met silencing in a mouse cell line decreased the ROS production, and an increase of ROS production by turning on the mTOR signaling pathway led to the migration of HSC out from mice BM (Tesio et al., 2011). In crayfish, a relationship between the cytokine Ast1 and ROS production also exists which is discussed in Paper I. These results indicate that a role of ROS signaling in hematopoietic regulation is conserved from invertebrates to vertebrates.

Objectives

The aim of this thesis is to provide a better understanding of the molecular mechanisms that regulate crustacean hematopoiesis. The freshwater crayfish, *Pacifastacus leniusculus* was used as a model in the studies, aiming to investigate how different factors, especially ROS signaling, Ast1, and the PVF/PVR signaling pathway are involved in controlling stem cell behavior during hematopoiesis. Moreover, the focus of the studies included in this thesis is about the role of extracellular TGase activity and the extracellular matrix in crayfish hematopoietic tissue for stem cell proliferation, differentiation and the release of mature hemocytes into the circulatory system.

Results and Discussion

Reactive oxygen species (ROS) affect transglutaminase activity and regulate hematopoiesis in a crustacean. (Paper I)

ROS serves as a commitment signal for balancing the proliferation and differentiation of progenitor cells in both mammal and *Drosophila* hematopoiesis (Bigarella et al., 2014; Owusu-Ansah and Banerjee, 2009). In the crayfish, *P. leniusculus,* we have shown that ROS acts as a short pulse signal, which rapidly increases in APC for 30-60 min after a LPS or laminarin injection, and then induces hemocyte production (Noonin et al., 2012). In this paper, the function of ROS in the regulation of hematopoiesis was further investigated in more detail.

The antioxidant N-acetylcysteine (NAC) was used to decrease the ROS level, both *in vivo* and *in vitro.* NAC serves as a scavenger for ROS in contrast to H_2O_2, which increases the ROS level. A decrease in ROS level in APC and HPT could be detected at 1 h after NAC injection in *in vivo* experiments. Moreover, lower level of ROS was observed in APC and HPT cells at 30 min after NAC was added into the culture medium *in vitro*. At 24 h after NAC injection, the ROS level in APC and HPT was restored to normal levels. A similar restoration of the ROS level was observed in cultured HPT cells after the addition of NAC to the culture for 30 min and when the cells were maintained in new culture medium for 24 h without NAC. The APC is a small part of the hematopoietic organ, and a high ROS level has been reported in this area (Noonin et al., 2012) and this was also shown to be the case in paper I. Consequently, the effect of NAC on ROS production was clearly observed in the APC. The high ROS level in this area might originate from high metabolic activity of the actively proliferating cells located in the APC, compared to cells in the rest of HPT, which are more differentiated and divide with a slower rate. An inhibition of ROS production in APC and HPT by NAC injection resulted in a lower total hemocyte number (THC) for at least 24 h, and then, the number of circulating hemocytes in these NAC injected animals was restored to normal levels later as compared to the control, (crayfish PBS injection). We have shown that LPS injection causes a dramatic decrease in hemocyte number and induces a rapid increase of ROS

production in the APC (Noonin et al., 2012). A combined injection of NAC and LPS resulted in inhibition of ROS production, and as a result a slower rate of new hemocyte release after this LPS injection was observed. These results confirm our earlier suggestion (Noonin et al., 2012) that the rapid increase in ROS production in APC may serve as a first signal to activate hematopoiesis and release hemocytes into circulation to prevent pathogen infection or to remove in this case LPS, a pathogen associated molecular pattern (PAMP).

In crayfish, Ast1, a hematopoietic growth factor, was purified from plasma and was used as a supplement to maintain the proliferation and differentiation of HPT cells in *in vitro* cell cultures (Söderhäll et al., 2005). Interestingly, a low ROS level in NAC treated cell cultures supplemented with crude Ast1 caused a lowered degree of cell spreading and cell migration. Migrating cells (spindle-shaped) were observed in control and H_2O_2 treated cells (to induce ROS production) in contrast to in NAC treated cells, which had a higher number of round cells. This result complies with the decrease of circulating hemocyte number after NAC injection *in vivo*. Furthermore, we showed co-localization of extracellular TGase and collagen IV on APC and HPT cell membranes and that high extracellular TGase activity was found in APC and HPT tissue cultures after NAC treatment. Again, these results confirm our previous report (Lin et al., 2008) that high extracellular TGase activity was found in undifferentiated cells (round cells), and that extracellular TGase activity is decreased in cells that are migrating out from the HPT tissue.

In short, we have found a possible role for ROS signaling to control extracellular TGase activity during hematopoiesis. A low ROS level caused an increase in extracellular TGase activity in both APC and HPT which provided a suitable environment for preventing progenitor cells from differentiation and to maintain the stem cells in the HPT tissue.

Role of astakine1 in regulating transglutaminase activity. (Paper II)

The injection of native purified Ast1 into crayfish results in an increase in the total number of circulating hemocytes (Söderhäll et al., 2005). TGase is one of the most abundant proteins in HPT and its activity has been studied during hematopoiesis (Junkunlo et al., 2016; Lin et al., 2008). The addition of crude Ast1 to HPT cell cultures results in decreased extracellular TGase activity (Lin et al., 2008), and in this paper, the role of Ast1 in regulating TGase activity was investigated in more detail. The effect of Ast1 on TGase

activity was studied using both endogenous TGase in a crayfish HPT cell extract, and also by using commercial guinea pig TGase. Then we found that Ast1 reduced enzymatic activity of both endogenous TGase and the commercial guinea pig TGase in a dose dependent manner. The kinetics of the inhibition experiments was investigated by varying concentrations of substrate and Ast1, and as a result Ast1 was shown to act as a non-competitive inhibitor of TGase activity. Furthermore, we examined the temperature optimum for crayfish TGase, and found that this enzyme functions in a very broad range of temperature from 4°C to 37°C. This was in contrast to the temperature range for commercial guinea pig TGase (Sirikharin R. personal communication). Crayfish are cold-blooded animals in contrast to mammals, and this broad temperature range for TGase may be of importance in order to enable clot formation also in cold water.

To investigate whether the inhibitory effect of Ast1 on TGase activity also could have an effect on clot formation, we investigated CP crosslinking by a gel formation assay. The clotting reaction was clearly reduced when native Ast1 was added into the reaction tube. This result suggests that Ast1 can inhibit plasma coagulation by interfering with TGase-mediated CP crosslinking. In addition, we could use antibodies against the ε-(γ-glutamyl) lysine bonds to show the presence of TGase mediated crosslinks between the CP molecules. Our results show that without the addition of Ast1, a stronger crosslinking ε-(γ-glutamyl) lysine bond signal between CP molecules was observed, in contrast to when Ast1 was added into the reaction. Again, these results confirm the inhibitory role of Ast1 in TGase-mediated CP crosslinking.

In summary, we have clarified the function for Ast1 in regulating TGase activity. Ast1 had an inhibitory effect on crayfish TGase activity as well as on rodent TGase enzyme activity and acted as a non-competitive inhibitor. Moreover, Ast1 reduced the TGase mediated crosslink bond formation of the crayfish CP protein. Since Ast1 is present in plasma, this may be a way to regulate and localize the clotting reaction during wounding or an infection, in order to avoid uncontrolled and widespread hemolymph coagulation. It is important here to clarify that TGase can have several more substrates and not only CP so the inhibitory effects of Ast1 on TGase activity may have more targets

PDGF/VEGF related receptor affects transglutaminase activity to control cell migration during crustacean hematopoiesis. (Paper III)

The function of the PDGF/PDGFR signaling pathway and its role in stem cell activity has been well characterized in vertebrates. However, very few studies have been performed in invertebrates and in crustaceans. In invertebrates, mainly PVF in *Drosophila* and Ast1 in crayfish are shown to act as hematopoietic growth factors. In *Drosophila*, the PVF/PVR signaling pathway is present and plays an important role in proliferation of larval hemocytes (Ratheesh et al., 2015). Therefore, we decided to investigate if the PVF/PVR pathway might exist and function in crayfish hematopoiesis. In this paper, the function of PVF/PVR signaling pathway in controlling stem cell behavior was studied in crayfish.

Four different sequences of PVR (Pl_PVR) isoforms were discovered in a crayfish transcriptome library, and these isoforms were expressed in several different tissues. Pl_PVR1 was highly expressed in mature hemocytes, both GCs and SGCs, but to a lower degree in HPT cells. The expression pattern of Pl_PVR3 is the same as Pl_PVR1, whereas Pl_PVR2 and Pl_PVR4 were abundantly expressed in most tissues. Furthermore, we found that RNAi knockdown of TGase in HPT cells resulted in an increase of Pl_PVR1 mRNA expression, but this increase varied a lot between animals due to unknown factors. However, this result may indicate a potential role of TGase in regulating the PVF/PVR signaling pathway in crayfish like in mammals (Zemskov et al., 2012). The full-length of Pl_PVR1 was cloned, the sequence submitted to GenBank and given the accession number KY444650. The protein domain structure of Pl_PVR1 is conserved in comparison to other PDGF/VEGF family protein. Unlike *Drosophila* PVR, the catalytic domain of Pl_PVR1 contains a nonspecific STYKc, which can phosphorylate serine/threonine/tyrosine amino acid residues. The multi-targeted inhibitor for a receptor tyrosine kinase named Sunitinib malate was used to inhibit the PVF/PVR downstream signaling pathway, in order to find putative functions of this pathway in crayfish HPT cells. We found that this substance at 1 μM and 10 μM induced a flat-shaped morphology in HPT cell culture. The flat-shaped cells had an increased cell surface area and appeared to be flat with several protrusions. This flat-shaped cell morphology was different from the "migrating" cell morphology (spindle-shaped cells), which previously was reported to appear after crude Ast1 addition as well as after TGase mRNA knockdown (Lin et al., 2008; Söderhäll et al., 2005). The total number of spreading cells (spindle-shaped and flat-shaped) increased in a dose dependent manner after Sunitinib malate treatment. Furthermore, crude Ast1 increased the effect of Sunitinib malate on cell spreading. The spreading morphology could be clearly observed at an earlier time point, at 24 h, and at lower concentration, 1 μM

Sunitinib malate treatment, in the presence of Ast1. In addition, after treatment with 5 μM Sunitinib malate, HPT cells were stimulated to migrate out of the tissue when this tissue was cultured *in vitro*. Moreover, an increase of β-tubulin immunoreactivity and stretching was observed in the flat-shaped cells, which probably promoted cell migration. Our previous results have shown a potential role of extracellular TGase activity in controlling cell spreading (Paper I and Lin et al., 2008). In this paper, a diverse function of TGase in controlling cell migration was found. Surprisingly, high level of extracellular TGase activity was found on the HPT cell surface in a dose dependent manner after Sunitinib malate treatment. In NHDF fibroblast cells, a stable interaction between PDGFR and integrin was reported in the presence of TGase (Zemskov et al., 2009), and the formation of a stable interaction between integrin-TGase-PDGFR promotes the receptor clustering and amplifies the downstream signaling. This activation induces a PDGFR-dependent cell response including cell proliferation and migration (Zemskov et al., 2009). A β-integrin was previously identified and cloned from hemocytes of crayfish (Holmblad et al., 1997). In addition, the regulatory region of crayfish TGase contains an integrin-binding site. Therefore, it is possible that β-integrin is involved in the regulation of TGase activity. Thus, we hypothesize that the accumulation of extracellular TGase on the crayfish HPT cell surface after Sunitinib malate treatment could occur through the association of TGase with integrin and PVR on HPT cell surface like in NHDF fibroblast cells (Zemskov et al., 2009). These results provide an additional role of extracellular TGase which is not only required to maintain cells in their undifferentiated stage but also to control HPT cell spreading through the PVR signaling pathway. This result is a bit surprising, and since then we have identified an additional TGase enzyme, this may be a result of activity of two different TGase enzymes.

In conclusion, we have shown that the PVF/PVR signaling pathway plays an important role in controlling hematopoietic progenitor cell behavior in crayfish. The inhibition of the PVF/PVR signaling pathway resulted in an increase of extracellular TGase activity at the HPT cell surface and induced cell migration.

Clotting protein – an extracellular matrix (ECM) protein involved in crustacean hematopoiesis. (Paper IV)

Clot formation is achieved by the crosslinking of plasma CP by endogenous TGase released from the hemocytes (Hall et al., 1999; Kopácek et al., 1993). The CP mRNA is dominantly expressed in the HPT, whereas it is hardly detected in hemocytes. In paper II we found that Ast1 can act as a regulator of clot formation by inhibiting crayfish TGase. In addition, we have earlier shown the important function of Ast1 and TGase in controlling stem cell

activity (Lin et al., 2008). For this reason, we decided to investigate a putative role of CP in hematopoiesis.

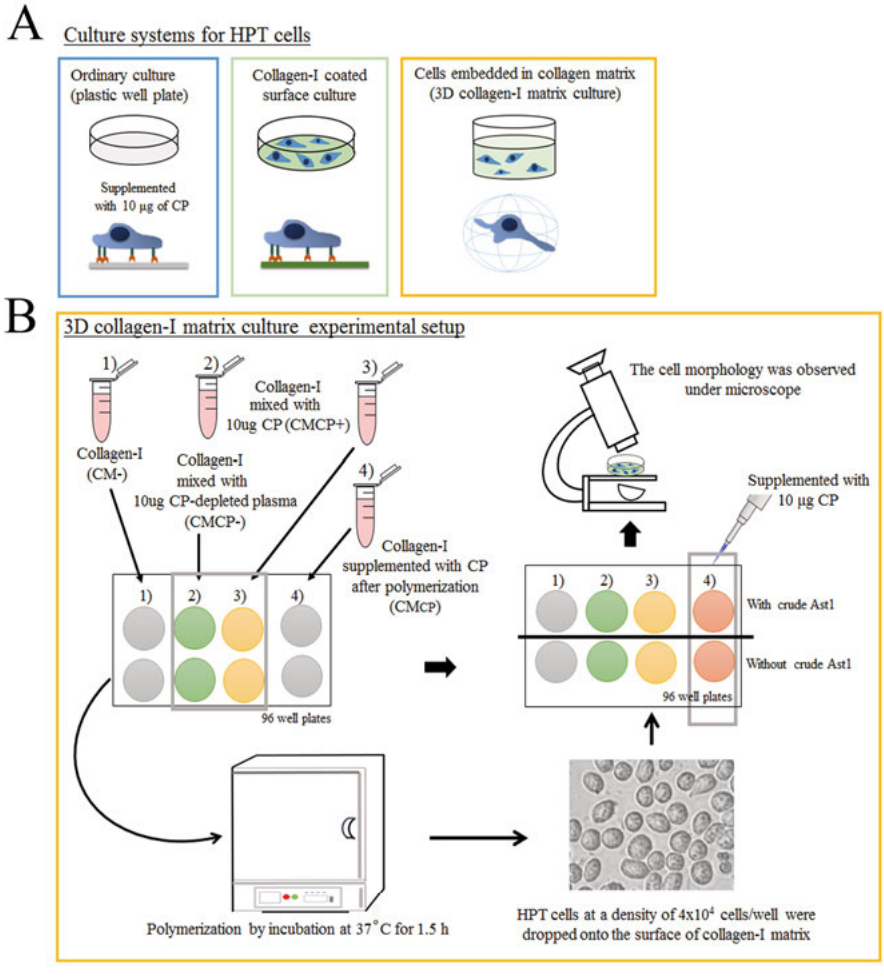

Figure 3. Experimental design for studying the effects of CP on HPT cell morphology. A) The addition of CP in HPT cell cultures were tested in three different cell culture systems; 1) normal culture (plastic well plates) supplemented with 10 µg of CP, 2) collagen-I coated surface, and 3) 3D collagen-I matrix gel culture. B) In 3D collagen-I matrix gel cultures, four different experimental groups were tested; 1) CM- (which consists of collagen I matrix without additional protein), 2) CMCP- (in which CP-depleted plasma was added to collagen-I solution and was allowed to slowly polymerize together with collagen-I), 3) CMCP+ (in which CP was added to collagen-I solution and was allowed to slowly polymerize together with collagen-I), and 4) CMcp (in which CP was added to the culture medium after collagen-I polymerization together with the HPT cells). The effect of Ast1 and CP on HPT cell morphology was determined by comparing the morphology of the cells cultured in the presence or absence of Ast1.

First we used antibodies towards CP and could observe the secretion and the formation of an extracellular filamentous network between cells in HPT cultures, especially when the cells started to spread. A higher amount of CP was found on the surface of round-cells than on the spreading cells in a similar manner as with extracellular TGase activity (Lin et al., 2008). Furthermore, co-localization of CP and extracellular TGase activity was detected on the HPT cell membranes and at the filamentous network between cells. This result suggests that CP may function as a part of an ECM in crayfish HPT. CP is a secreted protein and to study the function of CP, purified CP was added into ordinary HPT cell cultures (in plastic well plates), as well as in collagen-I coated wells, and in a 3D-collagen-I culture, where cells are cultured in a matrix made of collagen-I (Figure 3A). The addition of CP into the HPT cultures in plastic wells or in collagen-coated surface had no effect to the HPT cell morphology. However, in a 3D-collagen-I culture there was a remarkable effect on cell morphology after the addition of CP, either within the matrix or as an addition at the top. This result indicates that the surrounding of the cultured cells is of high importance for controlling cell behavior. We have shown earlier that culture in a 3D-collagen-I matrix may in fact help the cells to survive and differentiate in culture (Noonin et al., 2012). Therefore, 3D-collagen-I matrix cultures were performed to mimic more closely the *in vivo* HPT environment. The important function of TGase in crosslinking of CP and the ECM component collagen-I, was examined in this study at two different conditions; 1) CP was incubated and polymerized with collagen-I before the HPT cells were added and cultured (CMCP+) and 2) CP was added together with the HPT cells (CMcp). The CP-depleted plasma was incubated and polymerized with collagen-I (CMCP-) and collagen-I alone without CP addition (CM-) served as controls in these experiments (Figure 3B).

In paper II, we have reported a function of Ast1 to inhibit TGase enzyme activity and TGase crosslinking activity. Therefore, in 3D-collagen-I culture experiments, the effect of Ast1 in inhibiting crosslink formation was investigated by supplementing the HPT cell cultures with crude Ast1 compared to without crude Ast1 addition. We found that without the addition of crude Ast1, HPT cells formed a monolayer of round-shaped cell in all treatment (CM-, CMCP-, CMCP+, and CMcp). However, when crude Ast1 was added into the HPT cultures (CMCP+ and CMcp), spreading of HPT cells was induced. Interestingly, we found that in CMCP+ cultures, HPT cells lost their spreading capacity after 48 h, suggesting that these spread cells revert into non-spread cells. The loss of spreading morphology of HPT in CMCP+ treatment may be due to the degradation of Ast1 after 48 h and then the TGase activity was restored. In treatment 2) CMcp, CP was added together with HPT cells and we suggest that endogenous TGase from HPT cells might facilitate the crosslinking between CP and collagen-I to form a stable

matrix. As a result, HPT cells in CMcp cultures were more elongated and stretched. The elongated cells formed a chain of cells throughout the surrounding ECM with the other cells or were connected with other colonies. The function of CP as a possible ECM component was further investigated by whole mount immunostaining with CP antibody. CP is clearly found around the surfaces of HPT cells or covers the lobules at the posterior part of HPT. Co-localization of CP and collagen type IV was also detected at the HPT cell surface after whole mount immunostaining.

In conclusion, these results indicate that CP functions as a part of ECM protein component in crayfish HPT. The polymerization of CP with other ECM proteins such as collagen may be required to maintain the proliferation and differentiation of HPT progenitor cells.

Concluding remarks

These studies include functional studies of ROS signaling, TGase, Ast1, the PVF/PVR pathway, and CP in regulation of hematopoiesis. We found that ROS acts as a committed signal in controlling proliferation/differentiation of progenitor cells by regulating the extracellular TGase activity and thereby the ECM structure. Low ROS signal was required to maintain progenitor cells inside of the HPT, as was an increased extracellular TGase activity on the cell membranes. A high TGase activity promoted the crosslink interaction between ECM and progenitor cells. Consequently, HPT cells were kept in a quiescent stage inside the tissue. We also found that CP not only functioned in the clotting reaction but also was an ECM component in crayfish HPT. TGase is a crosslinking enzyme, which have several substrates including CP and collagen. Moreover, co-localization of CP and collagen with extracellular TGase was observed on HPT cell surfaces. We found that Ast1 had an inhibitory effect of TGase activity by interfering with the formation of ε-(γ-glutamyl) lysine bond. Thus, Ast1 might interfere with the TGase mediated CP crosslinking in HPT and as a result an unstable interaction between progenitor cells and ECM was evident. An opposite function of extracellular TGase activity was shown in this study in which a high TGase activity could stimulate cell migration when the PVR signaling pathway was inhibited. The PVR downstream signaling pathway inhibition caused accumulation of extracellular TGase on the HPT cell membrane. One hypothesis may be that this increase in extracellular TGase is necessary for an interaction between β-integrin and PVR on the HPT cell surfaces. However the precise reason for this link between TGase activity and the PVR signaling still needs further investigations.

Svensk sammanfattning

Sötvattenskräftan, *Pacifastacus leniusculus*, har under flera decennier använts som en modell för att studera immunförsvar och bildning av blodkroppar, hematopoiesis. Kräftor, liksom insekter hör till gruppen leddjur som är den ojämförligt artrikaste djurgruppen på jorden. Dessa djur, och andra ryggradslösa djur är huvudsakligen beroende av ett medfött immunförsvar, eftersom de saknar ryggradsdjurens adaptiva antikroppsförsvar. Det är framförallt djurens blodkroppar eller hemocyter som cirkulerar runt i kroppen i den s.k. hemolymfan och där spelar viktiga roller i detta försvar. Därför är studier av blodcellsproduktion eller så kallad hematopoiesis viktiga för att ge ökad förståelse för hur djuren klarar av att hålla en tillräcklig nivå på antalet blodkroppar i cirkulationen, men också för att ytterligare förstå hur det medfödda immunförsvaret fungerar. Arbetet med denna avhandling syftar till att undersöka effekterna av bland annat reaktiva syreradikaler (ROS) som signalmolekyler med betydelse för blodkroppsbildningen, men också betydelsen av cytokinet astakin1 (Ast1), samt signalering via PDGF/VEGF-liknande receptorer. Främst ligger fokus i studierna på betydelsen av den extracellulära miljön i den blodkroppsbildande vävnaden, HPT, och där specifikt på enzymet transglutaminas (TGas) som medverkar till att koppla ihop olika proteiner till en stabil matrix som håller de omogna blodkroppsprekursorerna på plats och möjliggör att signaler mellan celler kan regleras.

Reaktiva syreradikaler, ROS, är normalt skadliga för celler men det har även visats sig att små korta pulser av hög ROS-nivå kan fungera som signal i celler för att påverka deras differentiering. Hur ROS påverkar blodkroppsbildningen hos kräftan studerades genom att använda en antioxidant kallad NAC för att hämma ROS-produktion. Det visade sig att en låg nivå av ROS resulterade i att blodkroppsbildningen fördröjdes avsevärt, och särskilt då kräftan utsattes för en simulerad infektion genom injektion av LPS, som är delar av bakteriecellväggar. Dessutom befanns en låg ROS-nivå i cellkulturer påverka enzymet TGase så att dess aktivitet ökade utanför cellerna och därmed hindrades cellerna från att röra sig. Genom att TGase och ett av de strukturella proteiner nämligen kollagen typ IV som bygger upp den extracellulära miljön, ECM, visade sig vara samlokaliserade på blodkroppsprekursorernas cellytor drog vi slutsatsen att ROS tjänar som en primalsignal för att kontrollera celldelning och differentiering av förstadier till blodkroppar genom att påverka extracellulär TGas-aktivitet. En låg ROS-nivå krävdes

för att hålla cellerna inne i vävnaden, medan en kort ROS-puls initierade ny frisättning av blodkroppar till cirkulationen. Ast1- och TGas-aktivitet har tidigare klarlagts som viktiga faktorer under hematopoiesis hos kräftor. I denna avhandling har vi kunnat visa en hämmande effekt av Ast1 på TGas-enzymaktivitet och på dess förmåga att koppla samman proteiner med kovalenta bindningar. TGase är ett betydelsefullt enzym i kräftans koaguleringsprocess, där det verkar genom att koppla ihop koaguleringsproteinet CP till stora gelartade nätverk som hindrar förlust av hemolymfa vid en skada eller infektion. Följaktligen påverkar Ast1 också koagulationsprocessen genom att hämma TGase-enzymets aktivitet. Detta är en helt ny upptäckt av Ast1's funktion, och dess roll kan vara att reglera koagulering i kräftans cirkulation så att den sker lokalt och bara där den behövs.

Vi fann även en roll för CP i den hematopoietiska vävnaden. Utsöndring av koagulationsproteinet (CP) och produktionen av CP-filamentnätverk mellan celler observerades i HPT-cellkulturer in vitro. Vidare inducerades spridning av dessa celler i närvaro av CP tillsammans med Astl i 3D-kollagen-1-kulturer. HPT-cellerna befanns vara mer långsträckta och de bildade cellkedjor genom den omgivande matrixen. I HPT-vävnaden var CP lokaliserad runt HPT-cellerna och således visade sig CP att vara en del av ECM och eventuellt fungera tillsammans med kollagen för att skapa en lämplig miljö för de blodkroppsbildande stamcellerna.

För att undersöka funktionen av signalering via PDGF/VEGF-liknande receptorer (PVF/PVR) i reglering av kräftans blodkropps-bildning användes Sunitinib-malat som är en molekyl som specifikt hämmar denna typ av tyrosinkinas-receptorer nedströms signaleringsvägen. Inhibering av denna väg resulterade i en dramatisk förändring av cellernas morfologi och resulterade i en ökad cellyta under cellodling. Tillsatsen av Ast1 i cellodlingarna in vitro förstärkte denna effekt. Följaktligen stimulerades cellmigration efter inhibering av PVF/PVR-signalvägen. Vidare hittades en hög extracellulär TGase-aktivitet på cellernas yta efter denna inhibering, och anledningen till detta diskuteras i avhandlingen.

Sammanfattningsvis ger arbetet i denna avhandling ny insikt i betydelsen av den extracellulära matrisens (ECM) och extracellulära TGas-aktivitetens roll vid kontroll av stamcellsaktivitet. Kunskapen från detta arbete betonar vikten av faktorer som speciellt ROS-signalering, Ast1, PVF/PVR-signalvägen och CP vid reglering av hematopoiesis.

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References

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2002. The Extracellular Matrix of Animals.

Andrae, J., Gallini, R., Betsholtz, C., 2008. Role of platelet-derived growth factors in physiology and medicine. Genes Dev. 22, 1276–1312.

Anthony, B., Link, D.C., 2014. Regulation of Hematopoietic Stem Cells by Bone Marrow Stromal Cells. Trends Immunol. 35, 32–37.

Arango Duque, G., Descoteaux, A., 2014. Macrophage Cytokines: Involvement in Immunity and Infectious Diseases. Front. Immunol. 5.

Baker, B.M., Chen, C.S., 2012. Deconstructing the third dimension – how 3D culture microenvironments alter cellular cues. J. Cell Sci. 125, 3015–3024.

Beck, G., 2014. Cytokines, Evolutionary Aspects and Functions☆, in: Reference Module in Biomedical Sciences. Elsevier.

Benmimoun, B., Haenlin, M., Waltzer, L., 2015. Blood cell progenitor maintenance: Collier barks out of the niche. Fly (Austin) 9, 160–164. Bigarella, C.L., Liang, R., Ghaffari, S., 2014. Stem cells and the impact of ROS signaling. Dev. Camb. Engl. 141, 4206–4218.

Benmimoun, B., Polesello, C., Haenlin, M., Waltzer, L., 2015. The EBF transcription factor Collier directly promotes Drosophila blood cell progenitor maintenance independently of the niche. Proc. Natl. Acad. Sci. U. S. A. 112, 9052–9057.

Benton, J.L., Kery, R., Li, J., Noonin, C., Söderhäll, I., Beltz, B.S., 2014. Cells from the Immune System Generate Adult-Born Neurons in Crayfish. Dev. Cell 30, 322–333.

Bigarella, C.L., Liang, R., Ghaffari, S., 2014. Stem cells and the impact of ROS signaling. Dev. Camb. Engl. 141, 4206–4218.

Boulais, P.E., Frenette, P.S., 2015. Making sense of hematopoietic stem cell niches. Blood 125, 2621–2629.

Bullock, C.M., Li, J.-D., Zhou, Q.-Y., 2004. Structural determinants required for the bioactivities of prokineticins and identification of prokineticin receptor antagonists. Mol. Pharmacol. 65, 582–588.

Chaga, O., Söderhäll, K., Lignell, M., 1995. The haemopoietic cells of the freshwater crayfish, Pacifastacus leniusculus. Anim Biol 4, 50–70.

Cerenius, L., Kawabata, S., Lee, B.L., Nonaka, M., Söderhäll, K., 2010. Proteolytic cascades and their involvement in invertebrate immunity. Trends Biochem. Sci. 35, 575–583.

Cerenius, L., Söderhäll, K., 2017. Crayfish immunity - Recent findings. Dev. Comp. Immunol.

Cerenius, L., Söderhäll, K., 2013. Variable immune molecules in invertebrates. J. Exp. Biol. 216, 4313–4319.

Chang, C.-C., Chang, H.-C., Liu, K.-F., Cheng, W., 2016. The known two types of transglutaminases regulate immune and stress responses in white shrimp, Litopenaeus vannamei. Dev. Comp. Immunol. 59, 164–176.

Chang, Y.-T., Lin, C.-Y., Tsai, C.-Y., Siva, V.S., Chu, C.-Y., Tsai, H.-J., Song, Y.-L., 2013. The New Face of the Old Molecules: Crustin Pm4 and Transglutaminase Type I Serving as RNPs Down-Regulate Astakine-Mediated Hematopoiesis. PLOS ONE 8, e72793.
Chaves da Silva, P.G., Benton, J.L., Sandeman, D.C., Beltz, B.S., 2013. Adult neurogenesis in the crayfish brain: the hematopoietic anterior proliferation center has direct access to the brain and stem cell niche. Stem Cells Dev. 22, 1027–1041.
Chen, M.-Y., Hu, K.-Y., Huang, C.-C., Song, Y.-L., 2005. More than one type of transglutaminase in invertebrates? A second type of transglutaminase is involved in shrimp coagulation. Dev. Comp. Immunol. 29, 1003–1016.
Chen, P.-H., Chen, X., He, X., 2013. Platelet-derived growth factors and their receptors: Structural and functional perspectives. Biochim. Biophys. Acta BBA - Proteins Proteomics 1834, 2176–2186.
Chen, Y., Chen, W.-C., Cheng, W., 2014. The second type of transglutaminase regulates immune and stress responses in white shrimp, Litopenaeus vannamei. Fish Shellfish Immunol. 37, 30–37.
Cheng, W., Tsai, I.-H., Huang, C.-J., Chiang, P.-C., Cheng, C.-H., Yeh, M.-S., 2008. Cloning and characterization of hemolymph clottable proteins of kuruma prawn (Marsupenaeus japonicus) and white shrimp (Litopenaeus vannamei). Dev. Comp. Immunol. 32, 265–274.
Chlon, T.M., Crispino, J.D., 2012. Combinatorial regulation of tissue specification by GATA and FOG factors. Dev. Camb. Engl. 139, 3905–3916.
Crozatier, M., Vincent, A., 2011. Drosophila: a model for studying genetic and molecular aspects of haematopoiesis and associated leukaemias. Dis. Model. Mech. 4, 439–445.
Diamond, G., Beckloff, N., Weinberg, A., Kisich, K.O., 2009. The Roles of Antimicrobial Peptides in Innate Host Defense. Curr. Pharm. Des. 15, 2377–2392.
Dreyfuss, J.L., Regatieri, C.V., Jarrouge, T.R., Cavalheiro, R.P., Sampaio, L.O., Nader, H.B., 2009. Heparan sulfate proteoglycans: structure, protein interactions and cell signaling. An. Acad. Bras. Cienc. 81, 409–429.
Eckert, R.L., Kaartinen, M.T., Nurminskaya, M., Belkin, A.M., Colak, G., Johnson, G.V.W., Mehta, K., 2014. Transglutaminase Regulation of Cell Function. Physiol. Rev. 94, 383–417.
Eliasson, P., Jönsson, J.-I., 2010. The hematopoietic stem cell niche: low in oxygen but a nice place to be. J. Cell. Physiol. 222, 17–22.
Gábor, E., Cinege, G., Csordás, G., Török, T., Folkl-Medzihradszky, K., Darula, Z., Andó, I., Kurucz, É., 2017. Hemolectin expression reveals functional heterogeneity in honey bee (Apis mellifera) hemocytes. Dev. Comp. Immunol. 76, 403–411.
Gattazzo, F., Urciuolo, A., Bonaldo, P., 2014. Extracellular matrix: a dynamic microenvironment for stem cell niche. Biochim. Biophys. Acta 1840, 2506–2519.
Glazer, L., Sagi, A., 2012. On the involvement of proteins in the assembly of the crayfish gastrolith extracellular matrix. Invertebr. Reprod. Dev. 56, 57–65.
Glazer, L., Shechter, A., Tom, M., Yudkovski, Y., Weil, S., Aflalo, E.D., Pamuru, R.R., Khalaila, I., Bentov, S., Berman, A., Sagi, A., 2010. A Protein Involved in the Assembly of an Extracellular Calcium Storage Matrix. J. Biol. Chem. 285, 12831–12839.
Grigorian, M., Hartenstein, V., 2013. Hematopoiesis and Hematopoietic Organs in Arthropods. Dev. Genes Evol. 223.
Grigorian, M., Liu, T., Banerjee, U., Hartenstein, V., 2013. The proteoglycan Trol controls the architecture of the extracellular matrix and balances proliferation

and differentiation of blood progenitors in the Drosophila lymph gland. Dev. Biol. 384, 301–312.
Hall, M., Wang, R., van Antwerpen, R., Sottrup-Jensen, L., Söderhäll, K., 1999. The crayfish plasma clotting protein: A vitellogenin-related protein responsible for clot formation in crustacean blood. Proc. Natl. Acad. Sci. U. S. A. 96, 1965–1970.
Heldin, C.H., Westermark, B., Wasteson, A., 1979. Platelet-derived growth factor: purification and partial characterization. Proc. Natl. Acad. Sci. U. S. A. 76, 3722–3726.
Holmblad, T., Thörnqvist, P.-O., Söderhäll, K., Johansson, M.W., 1997. Identification and cloning of an integrin β subunit from hemocytes of the freshwater crayfish Pacifastacus leniusculus. J. Exp. Zool. 277, 255–261.
Holmes, D.I., Zachary, I., 2005. The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. Genome Biol. 6, 209.
Hsiao, C.-Y., Song, Y.-L., 2010. A long form of shrimp astakine transcript: molecular cloning, characterization and functional elucidation in promoting hematopoiesis. Fish Shellfish Immunol. 28, 77–86.
Huang, C.-C., Sritunyalucksana, K., Söderhäll, K., Song, Y.-L., 2004. Molecular cloning and characterization of tiger shrimp (Penaeus monodon) transglutaminase. Dev. Comp. Immunol. 28, 279–294.
Jagannathan-Bogdan, M., Zon, L.I., 2013. Hematopoiesis. Dev. Camb. Engl. 140, 2463–2467.
Jemaà, M., Morin, N., Cavelier, P., Cau, J., Strub, J.M., Delsert, C., 2014. Adult somatic progenitor cells and hematopoiesis in oysters. J. Exp. Biol. 217, 3067–3077.
Jia, Z., Kavungal, S., Jiang, S., Zhao, D., Sun, M., Wang, L., Song, L., 2016. The characterization of hematopoietic tissue in adult Chinese mitten crab Eriocheir sinensis. Dev. Comp. Immunol. 60, 12–22.
Jiang, X., Couchman, J.R., 2003. Perlecan and Tumor Angiogenesis. J. Histochem. Cytochem. 51, 1393–1410.
Jiravanichpaisal, P., Lee, B.L., Söderhäll, K., 2006. Cell-mediated immunity in arthropods: Hematopoiesis, coagulation, melanization and opsonization. Immunobiology 211, 213–236.
Johansson, M.W., Keyser, P., Sritunyalucksana, K., Söderhäll, K., 2000. Crustacean haemocytes and haematopoiesis. Aquaculture 191, 45–52.
Johansson, M.W., Söderhäll, K., 1985. Exocytosis of the prophenoloxidase activating system from crayfish haemocytes. J. Comp. Physiol. B 156, 175–181.
Jones, R.A., Kotsakis, P., Johnson, T.S., Chau, D.Y.S., Ali, S., Melino, G., Griffin, M., 2005. Matrix changes induced by transglutaminase 2 lead to inhibition of angiogenesis and tumor growth. Cell Death Differ. 13, 1442–1453.
Jorieux, S., Fressinaud, E., Goudemand, J., Gaucher, C., Meyer, D., Mazurier, C., 2000. Conformational changes in the D' domain of von Willebrand factor induced by CYS 25 and CYS 95 mutations lead to factor VIII binding defect and multimeric impairment. Blood 95, 3139–3145.
Jung, S.-H., Evans, C.J., Uemura, C., Banerjee, U., 2005. The Drosophila lymph gland as a developmental model of hematopoiesis. Dev. Camb. Engl. 132, 2521–2533.
Junkunlo, K., Söderhäll, K., Söderhäll, I., Noonin, C., 2016. Reactive Oxygen Species Affect Transglutaminase Activity and Regulate Hematopoiesis in a Crustacean. J. Biol. Chem. 291, 17593–17601.
Kawabata, S., 2010. Immunocompetent Molecules and Their Response Network in Horseshoe Crabs. SpringerLink 122–136.

Khadilkar, R.J., Ray, A., Chetan, D.R., Sinha, A.R., Magadi, S.S., Kulkarni, V., Inamdar, M.S., 2017. Differential modulation of the cellular and humoral immune responses in Drosophila is mediated by the endosomal ARF1-Asrij axis. Sci. Rep. 7, 118.

Kida, M., Souri, M., Yamamoto, M., Saito, H., Ichinose, A., 1999. Transcriptional Regulation of Cell Type-specific Expression of the TATA-less A Subunit Gene for Human Coagulation Factor XIII. J. Biol. Chem. 274, 6138–6147.

Kipryushina, Y.O., Yakovlev, K.V., Odintsova, N.A., 2015. Vascular endothelial growth factors: A comparison between invertebrates and vertebrates. Cytokine Growth Factor Rev. 26, 687–695.

Kopácek, P., Hall, M., Söderhäll, K., 1993. Characterization of a clotting protein, isolated from plasma of the freshwater crayfish Pacifastacus leniusculus. Eur. J. Biochem. 213, 591–597.

LeCouter, J., Zlot, C., Tejada, M., Peale, F., Ferrara, N., 2004. Bv8 and endocrine gland-derived vascular endothelial growth factor stimulate hematopoiesis and hematopoietic cell mobilization. Proc. Natl. Acad. Sci. U. S. A. 101, 16813–16818.

Li, F., Xu, L., Gai, X., Zhou, Z., Wang, L., Zhang, H., Gai, Y., Song, L., Yu, J., Liang, C., 2013. The involvement of PDGF/VEGF related factor in regulation of immune and neuroendocrine in Chinese mitten crab Eriocheir sinensis. Fish Shellfish Immunol. 35, 1240–1248.

Liang, G.-F., Liang, Y., Xue, Q., Lu, J.-F., Cheng, J.-J., Huang, J., 2015. Astakine LvAST binds to the β subunit of F1-ATP synthase and likely plays a role in white shrimp Litopeneaus vannamei defense against white spot syndrome virus. Fish Shellfish Immunol. 43, 75–81.

Lin, X., Kim, Y.-A., Lee, B.L., Söderhäll, K., Söderhäll, I., 2009. Identification and properties of a receptor for the invertebrate cytokine astakine, involved in hematopoiesis. Exp. Cell Res. 315, 1171–1180.

Lin, X., Novotny, M., Söderhäll, K., Söderhäll, I., 2010. Ancient cytokines, the role of astakines as hematopoietic growth factors. J. Biol. Chem. 285, 28577–28586.

Lin, X., Söderhäll, I., 2011. Crustacean hematopoiesis and the astakine cytokines. Blood 117, 6417–6424.

Lin, X., Söderhäll, K., Söderhäll, I., 2008. Transglutaminase activity in the hematopoietic tissue of a crustacean, Pacifastacus leniusculus, importance in hemocyte homeostasis. BMC Immunol. 9, 58.

Lord, M.S., Chuang, C.Y., Melrose, J., Davies, M.J., Iozzo, R.V., Whitelock, J.M., 2014. The role of vascular-derived perlecan in modulating cell adhesion, proliferation and growth factor signaling. Matrix Biol. J. Int. Soc. Matrix Biol. 35, 112–122.

Ludin, A., Gur-Cohen, S., Golan, K., Kaufmann, K.B., Itkin, T., Medaglia, C., Lu, X.-J., Ledergor, G., Kollet, O., Lapidot, T., 2014. Reactive Oxygen Species Regulate Hematopoietic Stem Cell Self-Renewal, Migration and Development, As Well As Their Bone Marrow Microenvironment. Antioxid. Redox Signal. 21, 1605–1619.

Mandal, L., Martinez-Agosto, J.A., Evans, C.J., Hartenstein, V., Banerjee, U., 2007. A Hedgehog- and Antennapedia-dependent niche maintains Drosophila haematopoietic precursors. Nature 446, 320–324.

Maningas, M.B.B., Kondo, H., Hirono, I., 2013. Molecular mechanisms of the shrimp clotting system. Fish Shellfish Immunol., Innate Immune System of Shrimp 34, 968–972.

Melrose, J., Roughley, P., Knox, S., Smith, S., Lord, M., Whitelock, J., 2006. The Structure, Location, and Function of Perlecan, a Prominent Pericellular Proteo-

glycan of Fetal, Postnatal, and Mature Hyaline Cartilages. J. Biol. Chem. 281, 36905–36914.
Miele, R., Lattanzi, R., Bonaccorsi di Patti, M.C., Paiardini, A., Negri, L., Barra, D., 2010. Expression of Bv8 in Pichia pastoris to identify structural features for receptor binding. Protein Expr. Purif. 73, 10–14.
Mondal, B.C., Shim, J., Evans, C.J., Banerjee, U., 2014. Pvr expression regulators in equilibrium signal control and maintenance of Drosophila blood progenitors. eLife 3, e03626.
Morrison, S.J., Scadden, D.T., 2014. The bone marrow niche for haematopoietic stem cells. Nature 505, 327–334.
Mouw, J.K., Ou, G., Weaver, V.M., 2014. Extracellular matrix assembly: a multiscale deconstruction. Nat. Rev. Mol. Cell Biol. 15, 771–785.
Naba, A., Hoersch, S., Hynes, R.O., 2012. Towards definition of an ECM parts list: An advance on GO categories. Matrix Biol. J. Int. Soc. Matrix Biol. 31, 371–372.
Nadalutti, C., Viiri, K.M., Kaukinen, K., Mäki, M., Lindfors, K., 2011. Extracellular transglutaminase 2 has a role in cell adhesion, whereas intracellular transglutaminase 2 is involved in regulation of endothelial cell proliferation and apoptosis. Cell Prolif. 44, 49–58.
Noonin, C., Lin, X., Jiravanichpaisal, P., Söderhäll, K., Söderhäll, I., 2012. Invertebrate hematopoiesis: an anterior proliferation center as a link between the hematopoietic tissue and the brain. Stem Cells Dev. 21, 3173–3186.
Nurminskaya, M.V., Belkin, A.M., 2012. Cellular Functions of Tissue Transglutaminase. Int. Rev. Cell Mol. Biol. 294, 1–97.
Osaki, T., Kawabata, S., 2004. Structure and function of coagulogen, a clottable protein in horseshoe crabs. Cell. Mol. Life Sci. CMLS 61, 1257–1265.
Owusu-Ansah, E., Banerjee, U., 2009. Reactive Oxygen Species prime Drosophila haematopoietic progenitors for differentiation. Nature 461, 537–541.
Parsons, B., Foley, E., 2013. The Drosophila PDGF and VEGF-Receptor Related (Pvr) Ligands Pvf2 and Pvf3 Control Hemocyte Viability and Invasive Migration. J. Biol. Chem. jbc.M113.483818.
Pennetier, D., Oyallon, J., Morin-Poulard, I., Dejean, S., Vincent, A., Crozatier, M., 2012. Size control of the Drosophila hematopoietic niche by bone morphogenetic protein signaling reveals parallels with mammals. Proc. Natl. Acad. Sci. U. S. A. 109, 3389–3394.
Persson, M., Vey, A., Söderhäll, K., 1987. Encapsulation of foreign particles in vitro by separated blood cells from crayfish, Astacus leptodactylus. Cell Tissue Res. 247, 409–415.
Ratheesh, A., Belyaeva, V., Siekhaus, D.E., 2015. Drosophila immune cell migration and adhesion during embryonic development and larval immune responses. Curr. Opin. Cell Biol., Cell adhesion and migration 36, 71–79.
Schneider, L., Cammer, M., Lehman, J., Nielsen, S.K., Guerra, C.F., Veland, I.R., Stock, C., Hoffmann, E.K., Yoder, B.K., Schwab, A., Satir, P., Christensen, S.T., 2010. Directional Cell Migration and Chemotaxis in Wound Healing Response to PDGF-AA are coordinated by the Primary Cilium in Fibroblasts. Cell. Physiol. Biochem. 25, 279–292.
Seita, J., Weissman, I.L., 2010. Hematopoietic stem cell: self-renewal versus differentiation. Wiley Interdiscip. Rev. Syst. Biol. Med. 2, 640–653.
Shibata, T., Kawabata, S., 2015. Transglutaminase in Invertebrates, in: Transglutaminases. Springer, Tokyo, pp. 117–127.

Sinenko, S.A., Shim, J., Banerjee, U., 2012. Oxidative stress in the haematopoietic niche regulates the cellular immune response in *Drosophila*. EMBO Rep. 13, 83–89.
Sirikharin, R., Junkunlo, K., Söderhäll, K., Söderhäll, I., 2017. Role of astakine1 in regulating transglutaminase activity. Dev. Comp. Immunol. 76, 77–82.
Smith, S.A., Travers, R.J., Morrissey, J.H., 2015. How it all starts: Initiation of the clotting cascade. Crit. Rev. Biochem. Mol. Biol. 50, 326–336.
Smith, V.J., Accorsi, A., Malagoli, D., 2016. Chapter 1 - Hematopoiesis and Hemocytes in Pancrustacean and Molluscan Models, in: The Evolution of the Immune System. Academic Press, pp. 1–28.
Söderhäll, I., 2016. Crustacean hematopoiesis. Dev. Comp. Immunol. 58, 129–141.
Söderhäll, I., 2013. Recent advances in crayfish hematopoietic stem cell culture: a model for studies of hemocyte differentiation and immunity. Cytotechnology 65, 691–695.
Söderhäll, I., Kim, Y.-A., Jiravanichpaisal, P., Lee, S.-Y., Söderhäll, K., 2005. An Ancient Role for a Prokineticin Domain in Invertebrate Hematopoiesis. J. Immunol. 174, 6153–6160.
Söderhäll, I., Bangyeekhun, E., Mayo, S., Söderhäll, K., 2003. Hemocyte production and maturation in an invertebrate animal; proliferation and gene expression in hematopoietic stem cells of Pacifastacus leniusculus. Dev. Comp. Immunol. 27, 661–672.
Söderhäll, K., Smith, V.J., 1983. Separation of the haemocyte populations of Carcinus maenas and other marine decapods, and prophenoloxidase distribution. Dev. Comp. Immunol. 7, 229–239.
Sricharoen, S., Kim, J.J., Tunkijjanukij, S., Söderhäll, I., 2005. Exocytosis and proteomic analysis of the vesicle content of granular hemocytes from a crayfish. Dev. Comp. Immunol. 29, 1017–1031.
Stofanko, M., Kwon, S.Y., Badenhorst, P., 2010. Lineage Tracing of Lamellocytes Demonstrates Drosophila Macrophage Plasticity. PLOS ONE 5, e14051.
Sugino, H., Terakawa, Y., Yamasaki, A., Nakamura, K., Higuchi, Y., Matsubara, J., Kuniyoshi, H., Ikegami, S., 2002. Molecular characterization of a novel nuclear transglutaminase that is expressed during starfish embryogenesis. Eur. J. Biochem. 269, 1957–1967.
Tesio, M., Golan, K., Corso, S., Giordano, S., Schajnovitz, A., Vagima, Y., Shivtiel, S., Kalinkovich, A., Caione, L., Gammaitoni, L., Laurenti, E., Buss, E.C., Shezen, E., Itkin, T., Kollet, O., Petit, I., Trumpp, A., Christensen, J., Aglietta, M., Piacibello, W., Lapidot, T., 2011. Enhanced c-Met activity promotes G-CSF-induced mobilization of hematopoietic progenitor cells via ROS signaling. Blood 117, 419–428.
Theopold, U., Krautz, R., Dushay, M.S., 2014. The Drosophila clotting system and its messages for mammals. Dev. Comp. Immunol., Drosophila Immunity 42, 42–46.
Theopold, U., Schmidt, O., Söderhäll, K., Dushay, M.S., 2004. Coagulation in arthropods: defence, wound closure and healing. Trends Immunol. 25, 289–294.
Van de Braak, C.B.T., Botterblom, M.H.A., Liu, W., Taverne, N., van der Knaap, W.P.W., Rombout, J.H.W.M., 2002. The role of the haematopoietic tissue in haemocyte production and maturation in the black tiger shrimp (Penaeus monodon). Fish Shellfish Immunol. 12, 253–272.
Vincent, A., Crozatier, M., 2010. Neither too much nor too little: reactive oxygen species levels regulate Drosophila hematopoiesis. J. Mol. Cell Biol. 2, 74–75.

Wang, R., Liang, Z., Hall, M., Söderhäll, K., 2001. A transglutaminase involved in the coagulation system of the freshwater crayfish, Pacifastacus leniusculus. Tissue localisation and cDNA cloning. Fish Shellfish Immunol. 11, 623–637.
Wang, Z., Griffin, M., 2012. TG2, a novel extracellular protein with multiple functions. Amino Acids 42, 939–949.
Wang, Z., Perez, M., Caja, S., Melino, G., Johnson, T.S., Lindfors, K., Griffin, M., 2013. A novel extracellular role for tissue transglutaminase in matrix-bound VEGF-mediated angiogenesis. Cell Death Dis. 4, e808.
Wasnik, S., Tiwari, A., Kirkland, M.A., Pande, G., 2012. Osteohematopoietic Stem Cell Niches in Bone Marrow. Int. Rev. Cell Mol. Biol. 298, 95–133.
Wood, W., Faria, C., Jacinto, A., 2006. Distinct mechanisms regulate hemocyte chemotaxis during development and wound healing in *Drosophila melanogaster*. J. Cell Biol. 173, 405–416.
Yarden, Y., Escobedo, J.A., Kuang, W.J., Yang-Feng, T.L., Daniel, T.O., Tremble, P.M., Chen, E.Y., Ando, M.E., Harkins, R.N., Francke, U., 1986. Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. Nature 323, 226–232.
Yeh, M.-S., Huang, C.-J., Leu, J.-H., Lee, Y.C., Tsai, I.-H., 1999. Molecular cloning and characterization of a hemolymph clottable protein from tiger shrimp (Penaeus monodon). Eur. J. Biochem. 266, 624–633.
Yeh, M.-S., Tsai, W.-L., Cheng, W., 2013. Identification and cloning of the second type transglutaminase from Litopenaeus vannamei, and its transcription following pathogen infection and in relation to the haemolymph coagulation. Fish Shellfish Immunol. 35, 1613–1623.
Yu, V.W.C., Scadden, D.T., 2016. Hematopoietic Stem Cell and Its Bone Marrow Niche. Curr. Top. Dev. Biol. 118, 21–44.
Yurchenco, P.D., 2011. Basement Membranes: Cell Scaffoldings and Signaling Platforms. Cold Spring Harb. Perspect. Biol. 3.
Zemskov, E.A., Loukinova, E., Mikhailenko, I., Coleman, R.A., Strickland, D.K., Belkin, A.M., 2009. Regulation of platelet-derived growth factor receptor function by integrin-associated cell surface transglutaminase. J. Biol. Chem. 284, 16693–16703.
Zemskov, E.A., Mikhailenko, I., Smith, E.P., Belkin, A.M., 2012. Tissue transglutaminase promotes PDGF/PDGFR-mediated signaling and responses in vascular smooth muscle cells. J. Cell. Physiol. 227, 2089–2096.
Zhang, C.C., Sadek, H.A., 2014. Hypoxia and metabolic properties of hematopoietic stem cells. Antioxid. Redox Signal. 20, 1891–1901.

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