Meat Science 119 (2016) 138–146

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Review

The role of matrix metalloproteinases in muscle and adipose tissue development and meat quality: A review

Sara Christensen, Peter P. Purslow *

Departamento de Tecnología y Calidad de los Alimentos, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Tandil, Argentina

ARTICLE INFO

Article history:
Received 3 November 2015
Received in revised form 18 April 2016
Accepted 19 April 2016
Available online 21 April 2016

Keywords:
Matrix metalloprotease
MMP
TIMP
Meat
Connective tissue
IMF
Adipogenesis
Development
Muscle growth
Meat toughness

ABSTRACT

Matrix metalloproteinases (MMPs) are a group of enzymes that degrade extracellular matrix components but are also important signaling molecules that regulate many biological processes including muscle, adipose and connective tissue development. Most recently it has been discovered that MMPs act as intracellular signaling molecules inducing gene expression and altering related proteins in the nucleus. Several single nucleotide polymorphisms of MMPs and their inhibitors are known to exist and most of the research on MMPs to date has focused on their activity in relation to human health and disease. Nevertheless there is a growing body of evidence identifying important roles of MMPs as regulators of myogenesis, fibrogenesis and adipogenesis. The aim of this review is to highlight the currently known functions of the MMPs that have a direct bearing on the deposition of meat components and their relationship with meat quality. Some central pathways by which these enzymes can affect the tenderness, the amount and type of fatty acids are highlighted.

Contents

* Corresponding author.
E-mail address: ppurslow@gmail.com (P.P. Purslow).

http://dx.doi.org/10.1016/j.meatsci.2016.04.025
0309-1740/

1. Introduction

The metzincin superfamily of metallo-endopeptidases includes serralysins, astacins, paplaysins, adamlysins (which comprise ADAMs and ADAMTs) and matrixins, also known as matrix metalloproteinases, or MMPs (Vandenbrouke & Libert, 2014). Metzincins are all zinc-dependent proteases whose activity is regulated in vivo by endogenous tissue inhibitors of metalloproteinases (TIMPs). In addition to general activities as proteases of extracellular proteins, several groups of metzincins are known to be important regulators of cell signaling at the cell-matrix interface. Extensive and comprehensive reviews exist on the structure and function of the general metalloproteinase clan and their role in normal tissue functions and disease, some of which are referenced in Table 1, together with previous reviews on the structure, composition, expression, activation, regulation and physiological/pathological roles of MMPs.

Historically, (e.g. Woessner, 1991) it was thought that MMPs principally act as enzymes that degrade structural components of the extracellular matrix (ECM), but newer evidence shows that MMPs are also involved in a wide range of extra- and intra-cellular signaling pathways. MMPs play a central role as regulator of the tissue microenvironment under physiological conditions during development and tissue remodeling or conditions contributing to tissue destruction (Shiomi, Lamaitre, Darmiento, & Okada, 2010).

In this review we will focus specifically on the functions of the matrix metalloproteinases which have obvious or potential relations with meat quality. The major themes in this regard are (i) those roles associated with myogenesis and growth of muscle tissue (ii) the role of MMPs in the competing processes of fibrogenesis and adipogenesis, and (iii) the degradation and turnover of the extracellular matrix components in skeletal muscle and how this could be manipulated to improve meat tenderness. Lastly, we will discuss variations in the activity of the MMP system between different muscles in the carcass, or variations caused by SNPs in the genes coding for MMPs as contributors to variations in meat quality.

Table 1
Selected reviews on MMPs, ADAMs and ADAMTS which provide detailed accounts of metalloproteinase structure, regulation, inhibition and review various aspects of known functions. This list is by no means exhaustive.

Reference	Title	Principal topics covered
Matrisian (1992)	The matrix-degrading metalloproteinases	MMP structure, specificity, regulation by TIMPs, normal extracellular roles, MMP as mediators of growth factors and cytokines.
Nagase et al. (2006)	Structure and function of the matrix metallo-proteinases and TIMPs	Structure of the MMPs, mechanism of activation, inhibitions by TIMPs, MMP activity in cardiovascular disease.
Verma and Hansch (2007)	Matrix metalloproteinases (MMPs): Chemical–biological function and (Q)SARs	Structure and substrate selectivity of MMPs, chemistry of artificial inhibitors, structure–activity relationships, clinical trials of MMP inhibitors.
Alameddine (2012)	Matrix metalloproteinases and skeletal muscle: A brief review	MMP roles in muscle development, muscle repair, myopathies and response to exercise/disuse atrophy.
Chen and Li (2009)	Role of matrix metalloproteinases in skeletal muscle	Role of MMPs in normal muscle development and injury repair.
Murphy (2010)	Fell-muir lecture: Metalloproteinases: From demolition squad to master regulators	Overview of MMPs and ADAMs function roles in ECM degradation and ectodomain shedding, involvement in disease and therapeutic approaches.
White (2003)	ADAMs: modulators of cell–cell and cell–matrix interactions	ADAMs as disintegrin, sheddases, roles in normal biology and pathology.
Huovila et al. (2005)	Shedding light on ADAM metalloproteinases	ADAMs as sheddases genetic manipulations of activities, signaling pathways downstream of sheddase activity.
Khokha et al. (2013)	Metalloproteinases and their natural inhibitions in inflammation and immunity	Structure and activity of MMPs, ADAMs and TIMPs, expression and regulation, roles in inflammation and immunity.
Shiomi et al. (2010)	Matrix metalloproteinases, a disintegrin and metalloproteinases, and a disintegrin and metalloproteinases with thrombospondin motifs in non-neoplastic disease.	Structure and characteristics of metalloproteinases and TIMPs, involvement in disease of the circulatory, respiratory and nervous systems, liver, kidneys, joints and muscular disease.
Kelwick et al. (2015)	The ADAMTS (A disintegrin and Metalloproteinases with thrombospondin motifs) family.	ADAMTS genes and evolution, structure localization and activity, gene knockout and polymorphism effects, roles in development and disease.
Kessenbrock et al. (2010)	Matrix metalloproteinases regulators of the tumor microenvironment.	MMP characteristics, regulation, in vivo functions, role in apoptosis and specifically involvement in many aspects of cancer cell migrations and tumor progression.

2. Structural and biological characteristics of MMPs

2.1. General structure and properties of MMPs

The principal structural features of MMPs, as well as ADAMs and ADAMTS, are summarized in Fig. 1 (From Khokha, Murthy, & Weiss, 2013). Matrisian (1992); Nagase, Visse, and Murphy (2006), and Verma and Hansch (2007) in their publications describe in more detail the basic structure of the MMPs and their differences according to their grouping.

Currently, 24 different types of MMP have been identified as expressed proteases among vertebrates (Ajay Kumar, Mamta, Alok, Kamlesh, Shanthy et al., 2010). At least 11 MMPs and 3 of the 4 known TIMPs have been shown to be expressed in bovine skeletal muscle (Balcerzak, Querengesser, Dixon, & Baracos, 2001).

A typical MMP has in its structure four functional domains; a signal peptide plus a pro-domain with a conserved cysteine switch motif, the catalytic domain, the hemopexin domain and a linker peptide of variable length (Nagase et al., 2006). Very roughly speaking, the pro-domain is involved in activation of the enzyme, variations in the catalytic relate to differences in the preferred substrates of the enzymes, and variations in the last two domains relate to variations in localization of the enzymes. Based on their structural characteristics, MMPs thus are classified into secreted-type MMPs or membrane-anchored types (MT-MMPs), of which there are only six (Shiomi et al., 2010). The MT-MMPs are inserted in the plasma membrane by a transmembrane segment or a glycosylphosphatidylinositol (GPI) anchoring sequence (Manello & Medda, 2012). Both MT-MMPs and MMPs are divided into subgroups according to substrate specificity (Shiomi et al., 2010). Thus, MMPs are grouped into collagenases, gelatinases, stromelysins, membrane type (MT) and others.

2.2. Expression, secretion and regulation of MMPs

The regulation of MMP activity can be at several different levels and involves the gene expression, activation of zymogens and inhibition of the enzymes by endogenous inhibitors (Manello & Medda, 2012). Hence, regulation of MMP activation and activity is a multi-faceted process that has many inputs and cross-connections to multiple signally pathways. Fig. 2 describes these events in relation to the normal extracellular activation of MMPs. The vast majority of current knowledge

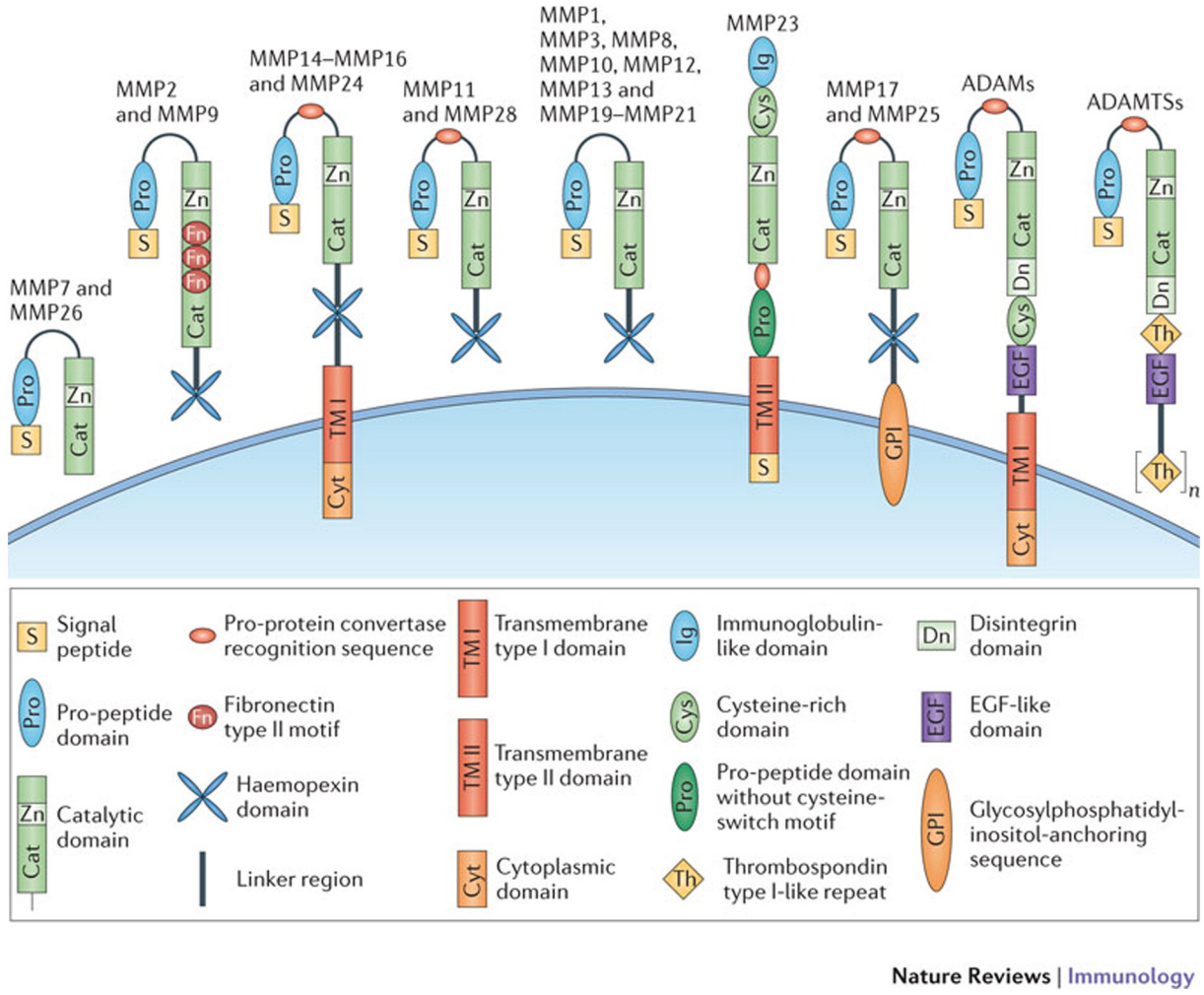

Fig. 1. General structure and domain characteristics of the metalloproteinases. Matrix metalloproteinases (MMPs) are a group of proteases which function in the extracellular environment of cells and degrade both matrix and non-matrix proteins. They are multi-domain proteins and their activities are regulated by tissue inhibitors of metalloproteinases (TIMPs). Reprinted by permission from Macmillan Published Ltd.: Nature Reviews/Immunology (Khokha et al., 2013).

about MMP activity focuses on their extracellular activity, although it is now known that some MMPs are active inside cells, as discussed in section 2.3.

MMP expression is tightly regulated at the transcriptional level and is induced by biochemical cell signaling by cytokines, chemokines, growth factors and chemical agents, as well as cell–cell or cell–matrix interactions, including mechanical stimulation signals from exercise (Lluri & Jaworski, 2005; Coronato, Laguens, & Girolamo, 2012). Some of the MMPs are transported to the cell surface in vesicles to be released extracellularly or to bind to the cell membrane.

The enzymes are synthesized as pro-MMPs; that is to say, in an inactive form inside the cell. Activation of the enzymes is an important step in the regulation of MMP activity (Manello, Tonti, & Papa, 2005; Rosenblum et al., 2007a). This is a process that normally occurs extracellularly (Manello & Medda, 2012) by other activated MMPs or by other serine proteinases, although the activation in some cases can be intracellular by pro-protein convertases such as furin or by aforementioned activators, or at the cell surface (for example, the formation of a MMP14–TIMP2–pro-MMP2 trimolecular complex activates pro-MMP2). Some MMPs have furin-like proprotein convertase recognition sequences in their structure at the end of the propeptide and may be activated intracellularly, and so will be secreted on the cell surface in active form or exert their action inside the cell.

The final step in the regulation of MMP activity is either their degradation or inhibition by endogenous inhibitors: the TIMPs (Manello & Gazzanelli, 2001; Troeberg & Nagase, 2007; Clark, Swingler, Sampieri, & Edwards, 2008). TIMPs are the major cellular inhibitors of the matrix metalloproteinase, exhibiting varying efficacy against different members. Four TIMPs are known (TIMP-1, 2, 3 and 4) but the ones with a greater role against MMPs are TIMP-1, TIMP-2 and TIMP-4 which inhibit different MMPs according to their location and substrate. These enzymes regulate MMP activity during tissue remodeling and possess other biological activities which may not be related to their inhibitory capacities (Baker, Edwards, & Murphy, 2002). The normal functions of MMPs depends on the balance between them and their physiological inhibitors (Kessenbrock, Plaks, & Werb, 2010). TIMPs also may be activators in some cases, e.g they can activate proMMP-2 and proMMP-9, the gelatinases responsible for proteolysis of denatured collagens.

2.3. MMPs are also active intracellularly

Localization of active MMPs inside the cell (in the nucleus, organelles or in the cytosol) represents an important aspect of the functions of MMPs that has heretofore been unappreciated. Manello and Medda (2012) summarize evidence from various studies showing MMP activity within cells. Although the roles of intracellular MMPs are not well defined, it appears that they are involved in cleaving and activating intracellular peptides, may induce gene expression, and can alter matrix proteins in the nucleus in healthy and disease conditions. So MMPs have a dual role as proteases of structural proteins and signaling

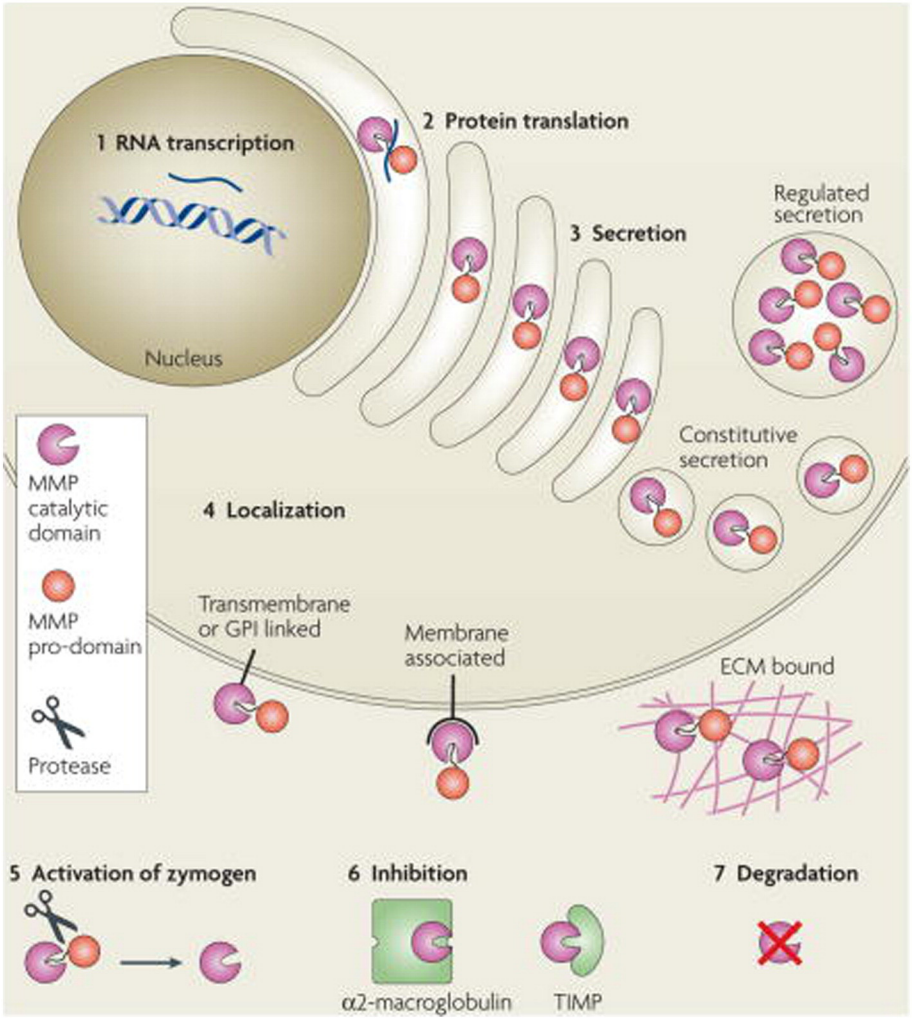

Fig. 2. MMPs function can be regulated at different levels 1 — RNA transcription (MMP expression) 2 — Protein translation 3 — Secretion 4 — Localization 5 — Activation of zymogen form 6 — Expression of their inhibitors like TIMP, α-macroglobin 7 — Protease degradation. It is important to consider the substrate availability and accessibility. This defines the degree of which MMP activity is used. Reprinted by permission from Macmillan Published Ltd.: Nature Reviews/Molecular Cell Biology (Page-McCaw et al., 2007)).

enzymes within a cell, just as they do extracellularly (Manello & Medda, 2012).

In skeletal muscle, Hadler-Olsen, Solli, Hafstad, Winberg, and Uhlin-Hansen (2015) detected MMP-2 (but not its sister gelatinase, MMP-9) concentrated in Z-lines of the sarcomeres, in the nuclear membrane, in mitochondria, and at the periphery of the myofibrils, probably in the sarcoplasmic reticulum or in T-tubuli. Furthermore, some results suggest that intracellular localization of MMPs in skeletal muscle fibers is active during normal homeostasis, and that their levels are affected by the intensity of physical activity (Hadler-Olsen et al., 2015). Intracellular substrates of MMP-2 in cardiomyocytes include α-actinin-1, desmin, myosin light chain-1, troponin I and titin, and so it has been inferred that MMP-2 probably contributes in regulation and repair of structural proteins in muscle producing contractile force (Sung et al., 2007b; Cauwe & Opdenakker, 2010; Ali, Fan, & Schulz, 2011; Hadler-Olsen et al., 2015). Cauwe and Opdenakker (2010) also showed the intracellular action of MMP-2 is capable of processing several mitochondrial proteins such as heat shock protein 75, −90α, and −90β and peroxiredoxin 5, linking the enzyme to regulation of protein folding and antioxidant activity.

However, Cha and Purslow (2010) showed that MMP activity in soleus muscle and heart muscle was mainly extracellular, despite the muscle cells containing high levels of pro-MMPs. They also observed that smaller diameter (probably oxidative) muscle fibers contain higher concentrations of pro-MMPs in rat soleus muscle. In contrast to this, Carmeli, Moas, Lennon, and Powers (2005) observed that high-intensity exercise promotes MMP-2 expression more in muscles with a high proportion of type II fibers (glycolytic fibers). According to Manello and Medda (2012), most of the enzyme MMP-26 is retained inside the cell which produces it, where it undergoes autocatalytic activation during high calcium influxes. Normal calcium levels within resting muscle fibers would probably maintain MMP-26 in an inactive state.

The recent observations of activity of MMPs inside the cell open up a new vision of their roles as both proteases and signaling molecules in the cell biology.

2.3.1. Nuclear localization of MMPs

MMPs and TIMPs have been shown to act in the nuclei of a wide range of human and animal cell types (Manello & Medda, 2012). In relation to skeletal muscle, direct evidence of perinuclear collagenase activity in myoblasts (Cha & Purslow, 2010) and in differentiated myotubes (Purslow, Archile-Contreras, & Cha, 2012) has been obtained by cell "in-situ" zymography. Although the possible functions and substrates of MMPs in the nucleus remain largely unknown, it is believed that these may include maintenance of nuclear matrix structure, chromatin remodeling, control of apoptosis, and regulation of cellular proliferation (Satyesh et al., 2014). It is known that TIMP-1 is also present in high concentrations in the nucleus and it is considered to be related to the regulation of MMPs involved in cell cycle inhibition. The novel intracellular and intranuclear functions of MMPs could be involved in cell

apoptosis by different mechanisms and forms of action. (Tonti, Manello, Cacci, & Biagioni, 2009; Manello & Medda, 2012; Hishikawa, Nakaki, & Frujii, 2000).

Clearly, as Manello and Medda (2012) suggest, the precise roles of MMPs in nuclear sorting and control of expression should be an important focus of future studies. At present, we can merely reflect that these metalloproteinases obviously have intercellular roles far beyond simple proteolysis.

3. Biological roles of MMPs during muscle, adipose and connective tissue development and their influence in meat quality

3.1. Activities of MMPs expected to influence meat quality

Two of the most desirable meat quality attributes for consumers are tenderness and juiciness. According to the past surveys of beef producers by National Cattlemen's Beef Association, marbling and tenderness were consistently identified as the top beef quality issues (McKenna et al., 2002; Garcia et al., 2008). Cooked meat tenderness is dominated by the contributions of myofibrillar proteins but also has a contribution from the amount and composition of the intramuscular connective tissue (IMCT). Intramuscular fat (marbling) levels are also associated with juiciness, flavor, tenderness and overall liking of the meat.

There is clear evidence of a strong involvement of MMPs and TIMPs in myogenesis, fibrogenesis and adipogenesis during muscle development. In early embryonic development, mesenchymal progenitor cells diverge into a myogenic lineage and a non-myogenic lineage. The non-myogenic lineage differentiates into adipocytes and fibroblasts and the myogenic cells into myocytes and satellite cells, as described by Miao et al. (2015) and summarized in Fig. 3.

During embryonic growth of muscles, adipogenesis and fibrogenesis (development of connective tissue forming cells) may be considered as a competitive process (Du et al., 2010; Miao et al., 2015). If the total density and proliferation of mesodermal progenitor cells are unaltered, enhancing adipogenic differentiation and reducing fibrogenic differentiation from progenitor cells is projected to increase the capacity for development of marbling and reduce the capacity for connective tissue deposition during post-natal growth (Du et al., 2013), and so may directly impact meat tenderness and juiciness. The balance between the differentiation of mesodermal progenitor cells into fibroblasts versus adipocytes is partly under the control of MMPs. Thus, the issues of fat deposition and connective tissue deposition in growing muscle are inextricably linked.

As the amount and maturity of the connective tissue in muscle contributes to the background toughness of beef, there is interest in minimizing the amount and strength of this component. As summarized by Purslow et al. (2012), a series of studies has targeted the possibility of reducing the abundance of mature cross-links in intramuscular collagen by increasing remodeling of the connective tissue by MMPs.

3.2. Biological roles of MMPs in relation with myogenesis

As the process of myocyte alignment in the embryonic development of muscle is patterned by interactions between the cells and extracellular matrix components, it should be no suprise that MMPs are implicated in this initial stage of myogenesis and in the fusion of uninucleate myocytes into multinucleate myotubes. Chen and Li (2009) demonstrate the involvement of MMP-1, MMP-2, MMP-9 and MT1-MMP in myogenesis and consider MMP-2 and MT1-MMP as major factors affecting the formation of myotubes in vitro. Alameddine (2012) observed MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-14 and MMP-16 in myogenic cells of various species. Other studies have also indicated an important role of MMP-2, MMP-7, MMP-9 and MT1-MMP in myotube formation (El Fahime, Torrente, Caron, Bresolin, & Tremblay, 2000; Lewis, Tippett, Sinanan, Morgan, & Hunt, 2000 and Caron, Asselin, Morel, & Tremblay, 1999) and Lluri and

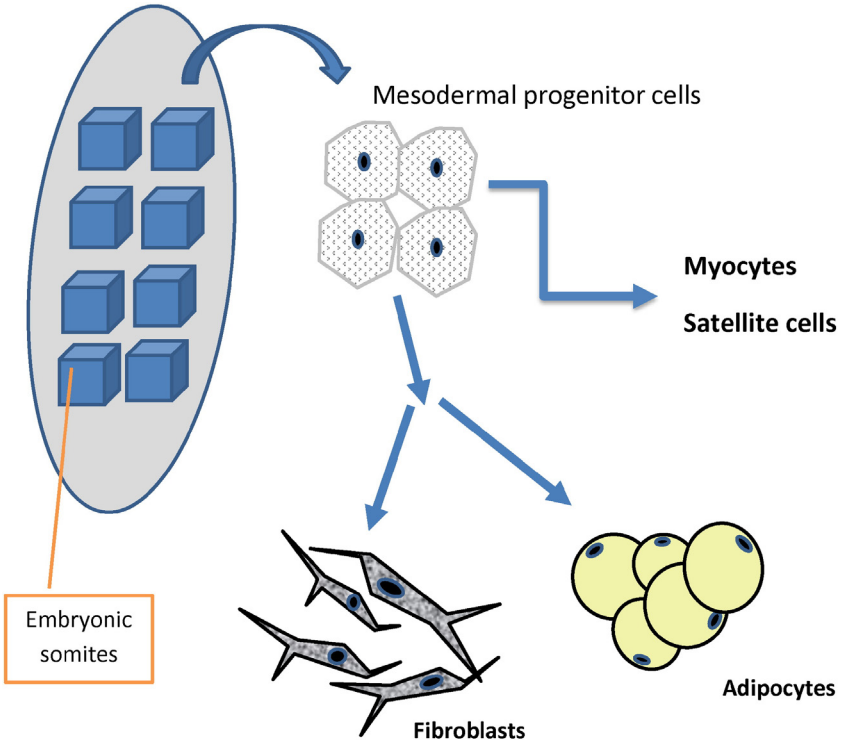

Fig. 3. Formation of major cell types in muscle from mesodermal cells in the embryonic somites. One line of progenitor cells forms satellite cells and the myocytes that fuse to form myotubes and subsequently muscle fibers. The other mesodermal progenitor line forms either fibroblasts (connective tissue-forming cells) or adipocytes. The differentiation into either fibroblasts or adipocytes is competitive; more of one equals less of the other. Hence, tipping the balance towards more adipocytes increases the potential for marbling and decreases the potential for IMCT deposition, and vice versa.

Jaworski (2005) confirmed that TIMP-2, MT1-MMP, and MMP-2 are up-regulated coincident with myogenesis.

3.3. MMPs in relation to post-natal muscle growth (hypertrophy)

MMPs are responsible for the degradation of intramuscular connective tissue to allow an appropriate increase in muscle fiber size when the muscle increases the size during growth of the animal (Purslow, 2005). Coupled with the activity of fibroblasts that synthesizes new components of the ECM (mainly collagen), a balance between degradation and synthesis of new collagen is generated. The amount and composition of the ECM are related to the functions of the growing muscle. The control of extracellular space surrounding the cell by MMPs allows cell migration, proliferation, tissue growth and repair as well as pathological tissue destruction in disease conditions. Different MMPs interact in specific ways with different extracellular matrix components. The collagenases (MMP-1, MMP-8, MMP-13 and MMP-18) digest interstitial collagen, types I, II and III, whereas the gelatinases A and B (MMP-2 and MMP-9 respectively) act on denatured collagen.

Animals in feedlots can generate much faster growth than animals grazing on pasture. Rapid growth requires rapid degradation and regeneration of the connective tissue layers separating growing muscle fibers. This rapid regeneration of connective tissue may diminish the formation of stable and insoluble mature crosslinking in comparison with the crosslinking of the meat of slower-growing animals that usually produce a firmer meat. Manipulation of the rate of remodeling of IMCT may be considered as a means to improve the tenderness of IMCT-rich muscles (Purslow et al., 2012).

3.4. MMP expression and activity varies between muscles and exogenous factors

In post-natal skeletal muscle tissue, both muscle cells and fibroblasts secrete MMPs and TIMPs (Purslow, 2002) but this expression of these enzymes is influenced by multiple factors like breed, age, sex, feed level, exercise and muscle fiber type. It is obvious that the balance between synthesis and degradation of ECM components is very different in different muscles, as the connective tissue content and architecture varies greatly between muscles with different biological functions. Archile-Contreras, Mandell, and Purslow (2010), demonstrated that fibroblasts isolated from 3 different muscles of the same cattle also have different MMP activity, again reflecting that the different amounts of connective tissue and architecture in these muscles influence in enzyme expression. Fibroblasts isolated from the longissimus, semitendinosus, and sternomandibularis muscles of the same beef animal showed significant differences in proliferative capacity and in the activity of MMP-2 that they express in a common basal environment. The level of expression of MMPs by muscle fibers appears to differ between fast glycolytic and slow oxidative muscle fiber types.

The function of MMPs is also influenced by reactive oxygen species (ROX) (Archile-Contreras & Purslow, 2011; Kessenbrock et al., 2010). The free radicals in the animal muscle are formed as a result of natural metabolism. Muscles have natural mechanisms through the expression of antioxidants to counteract the action of ROX, but when the balance is altered by diet, stress, or environmental pollution conditions, oxidative stress occurs. This oxidative stress can activate pro-MMPs. Oxidants such HOCL and ONOO– have the ability to activate pro-MMPs by reacting with the cysteine switch in the pro-peptide. Hence, ROX can affect the activity of intracellular MMPs. Vitamins E and C can counter the negative effects of oxidative stress on new collagen synthesis (Archile-Contreras, Cha, Mandell, Miller, & Purslow, 2011), but again it is interesting to note that the effects of these vitamins on the expression and activity of MMP-2 and the synthesis of new collagen by fibroblasts differs between fibroblast isolated from different muscles. Vitamin C is an essential cofactor in the divalent cross-link formation in newly synthesized collagen (Bailey & Light, 1989).

The presence of the stress hormone epinephrine also can influence MMP expression by myoblasts and fibroblasts (Cha & Purslow, 2011) and the beta-agonist growth promoter ractopamine has the effect of increasing extracellular MMP-2 activity in myoblasts and fibroblasts, counterbalanced by the increased presence of TIMP-1 (Cha & Purslow, 2012) in addition to its major effect of increasing muscle growth by altering the balance between syntheses and degradation of myofibrillar proteins.

3.5. Role of MMPs in adipogenesis and fatty acid composition

Intramuscular fat is located in the intramuscular connective tissue and, again, remodeling of the IMCT by MMPs and synthesis of new ECM components by fibroblasts in necessary for the growth of fat deposits between and within muscle fascicles. In addition to factors coming from the ECM environment surrounding the muscle cells, several MMPs are expressed by pre-adipocytes and adipocytes, especially MMP-2 and MMP-9 (Bouloumie, Sengenes, Portolan, Galitzky, & Lafontan, 2001; Van Hul, Lupu, Dresselaers, Buyse, & Lijnen, 2012).

Intramuscular fat content and fatty acid composition have a large influence on the palatability of meat (Scollan et al., 2014; Lee et al., 2007). Red meat has a high concentration of saturated fatty acids and low concentration of the polyunsaturated fatty acids considered beneficial to human health, and so it is important to identify the genes influencing muscle lipid profile (Dunner, Sevane, Garcia, Leveziel, et al., 2013b). The fatty acid composition of intramuscular fat differs between breeds and nutritional factors only have a little influence on this, so indicating that metabolism of fatty acids is partially under genetic control.

Chavey et al. (2003) clearly demonstrate that MMPs are involved in modulations of adipocyte differentiation. Because adipocytes and fibroblasts (connective tissue-forming cells) share common embryonic progenitor cells (mesenchymal cells) the factors promoting adipogenesis can result in more adipocytes and less fibroblasts (Miao et al., 2015). This may result in a greater potential to develop more marbling and a smaller potential to form connective tissue in the muscles of animals and influence in the tenderness. However, in a comparison of Nellore and Angus cattle breeds, Martins et al. (2015) found a higher marbling in the longissimus muscle in the Angus versus Nellore cattle, but no significant differences in the content of collagen. This apparent contradiction may be due to the very different growth rates between the two breeds. In faster-growing Angus cattle, the expression of MMPs, TIMP-1, collagen I and collagen III were all higher than in the Nellore cattle. The increased intramuscular fat in the longissimus of the Angus cattle may be due to hypertrophy of adipocytes during post-natal growth rather than a greater number of adipocytes produced during development.

O'Hara, Lim, Mazzati, and Trayhurn (2009) consider that expression MMP-1, MMP-3, MMP-9, MMP-10, MMP-12 and MMP-19 have a relation with the adipose tissue deposition. In contrast, Van Hul et al. (2012) showed that deficiency of MMP-2 is associated with impaired adipose development, whereas in their study MMP-9 expression had no significant relation to adipose tissue development. It has been shown that the inhibition of MMP decreases adiposity in mice and reduces the adipogenesis in 3T3-L1 and 3T3-F442A cells, and in vitro it has also been shown that specific inhibition of MMP-9 reduces adipogenesis in humans (Bourlier et al., 2005).

The fact that adipogenic and fibrogenic cells share immediate common progenitor cells provides an opportunity to manipulate the differentiation of progenitor cells to favor adipogenesis.

4. Polymorphisms and expression of MMPs and TIMPs

In cattle, several single nucleotide polymorphisms (SNPs) have been identified from genome sequencing and are utilized as molecular markers. SNPs in candidate genes are used to determine possible

phenotypic variations of the carcass traits in meat quality characteristics (Buchanan et al., 2002).

A polymorphism is a genetic variant that appears within genes in above 1% of a population and represents natural sequence variants which are likely responsible for variations in phenotype. SNPs can be causative or not, depending on their location. They are causative when the SNP is in a non-coding region and affects the amount or activity of the enzyme, or if the SNP is in a coding region and so produces changes in the amino acid sequence and hence the identity of the protein. In the bovine genome 2.3 million putative SNPs have been identified, but only around 123,000 of these were identified from regions with deep sequence coverage of a high quality and with high probability of being validated. (Williams et al., 2009; Jorgenson & Witte, 2006). SNPs occur at a frequency of about one SNP per 500 bp in and cattle (Heaton et al., 2001).

Many of the SNPs identified in cattle have been associated with variations in the carcass characteristics, growth rate and meat quality (Dunner, Sevane, Garcia, Cortes, et al., 2013a). Investigations by Dunner et al. (2013b) identified SNPs in genes related to adipogenesis and lipid profile in meat from 15 European cattle breeds. Among candidate genes analyzed, they found SNPs in MMP-1 associated with large effects on the fatty acid profile, specifically on docosahexaenoic acid (22:6 n-3) and conjugated linoleic acid (CLA) content. The SNPs ss77831914 and ss77831916 affect the amount of CLA, and SNP ss77831924 affects decosahexaenoic acid. A polymorphism in the MMP-1 gene was found that influenced the activity of the m-calpain and was associated with meat tenderness. We consider that further examination of SNPs in genes for MMPs and TIMPs could help to assess the role of these enzymes in the genetic control of muscle development and meat quality. A large number of SNPs in genes of MMPs and TIMPs have been entered in databases; the question of interest is if any of these have effects on the qualities of meat.

SNPs in MMPs and TIMPs may affect the amount and composition of the fat and the connective tissue content and the cross-linking in the muscles from animals. The identity of the SNPs in the MMPs and TIMPs probably differs between breeds and their effects may vary with feeding, age, sex, time of year of slaughter of animals. As mentioned above, SNPs are natural variations which arise between animals of the same breed.

5. Concluding remarks and outlook

It is known that the MMPs play a major role in ECM remodeling. Nevertheless, numerous recent studies have uncovered other multiple and divergent roles of these enzymes in two areas; as proteases of intracellular structural proteins and as signaling enzymes within a cell, just as they act extracellularly (Malemud, 2006; Manello & Medda, 2012). MMPs have significant cell-signaling and gene regulation activities during animal growth and ultimately impact meat quality, as schematically shown in Fig. 4.

MMPs are involved in the remodeling of IMCT to allow an increase in size of muscle fibers during post-natal growth. Increasing the rate of this continuous turnover of the connective tissue may suppress development of mature cross-links that are associated with tougher meat.

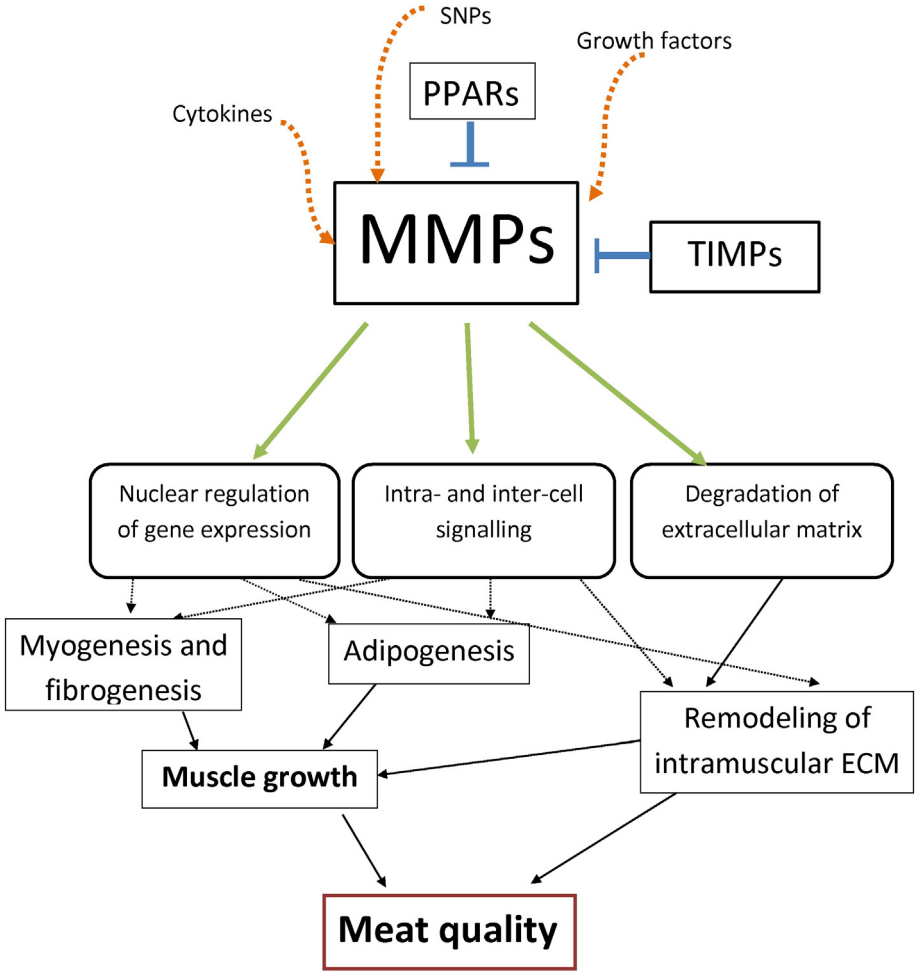

Fig. 4. Schematic diagram representing the main pathways by which MMPs and their inhibitors affect meat quality. PPARs (peroxisome proliferator-activated receptors) are nuclear receptor proteins that regulate gene expression. All other symbols and topics are described in the text.

MMPs influence the maturation of adipocytes and their lipid profiles and the preferred development of intramuscular connective tissue generates lower intramuscular fat and vice versa (Du et al., 2010). In terms of the effects of MMPs on the turnover of intramuscular connective tissue; as collagen content varies between muscles, we speculate that the associations between MMP/TIMP activity and meat quality will most probably vary between different muscles, and the influence of hormones and growth factors will also have variable effects, at least in the level of association.

Given that myogenic, adipogenic, and fibrogenic cells are derived from a common pool of mesenchymal progenitor cells, the manipulation of progenitor cell differentiation is an opportunity to enhance lean growth, reduce connective tissue accumulation, and alter fat deposition, with the objective of improving the efficiency and quality of meat production (Du et al., 2013). Less fibrogenic differentiation may reduce intramuscular connective tissue deposition, thereby decreasing background toughness of beef. It has been suggested that variations in maternal nutrition during fetal development may be a form of "fetal programing" to improve meat production (Du, Wang, Fu, Yang, & Zhu, 2015).

Although SNPs for MMPs and TIMPS are well-documented in beef cattle, there is sparse information on their effects on phenotypic aspects. As it is known that SNPs in MMPs affect the fatty acid profile, this can be expected to impact meat tenderness and juiciness. We can also postulate that SNPs in MMPs may affect the activity of the enzymes and this may also impact meat toughness.

Acknowledgments

The support of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) to SC is gratefully acknowledged. This work was also supported by Fondo para la Investigación Científica y Tecnológica (FONCyT) grant no. PICT 2013-3292.

References

Alameddine, H. S. (2012). Matrix metalloproteinases in skeletal muscles: Friends or foes? *Neurobiology of Disease*, *48*(3), 508–518.

Ajay, Kumar C., Mamta, S., Alok, C. B., Kamlesh, A., Shanthy, S., & Ravi, M. (2010). Genetic polymorphisms of matrix metalloproteinases and their inhibitors in potentially malignant and malignant lesions of the head and neck. *Journal of Biomedical Science*, *17*(1), 10.

Ali, M. A., Fan, X., & Schulz, R. (2011). Cardiac sarcomeric proteins: Novel intracellular targets of matrix metalloproteinase-2 in heart disease. *Trends in Cardiovascular Medicine*, *21*, 112–118.

Archile-Contreras, A. C., & Purslow, P. P. (2011). Oxidative stress may affect meat quality by interfering with collagen turnover by muscle fibroblasts. *Food Research International*, *44*, 582–588.

Archile-Contreras, A., Cha, M., Mandell, I., Miller, S., & Purslow, P. P. (2011). Vitamins E and C may increase collagen turnover by intramuscular fibroblasts; potential for improved meat quality. *Journal of Agricultural and Food Chemistry*, *59*, 608–614.

Archile-Contreras, A. C., Mandell, I. B., & Purslow, P. P. (2010). Phenotypic differences in matrix metalloproteinase 2 activity between fibroblasts from three bovine muscles. *Journal of Animal Science*, *88*, 4006–4015.

Bailey, A. J., & Light, N. D. (1989). *Connective tissue in meat and meat products*. London, UK: Elsevier Applied Science.

Baker, A. H., Edwards, D. R., & Murphy, G. (2002). Metalloproteinases inhibitors: Biological actions and therapeutic opportunities. *Journal of Cell Science*, *115*, 3719–3727.

Balcerzak, D., Querengesser, L., Dixon, W. X., & Baracos, V. E. (2001). Coordinate expression of matrix-degrading proteinases and their activators and inhibitors in bovine skeletal muscle. *Journal of Animal Science*, *79*, 94–107.1120472.

Bouloumie, A., Sengenes, C., Portolan, G., Galitzky, J., & Lafontan, M. (2001). Adipocyte produces matrix metalloproteinases 2 and 9: Involvement in adipose differentiation. *Diabetes*, *50*(9), 2080–2086.

Bourlier, V., Zakaroff-Girard, A., De Barros, S., Pizzacalla, C., Durand de Saint Front, V., Lafontan, M., ... Galitzky, J. (2005). Protease inhibitor treatments reveal specific involvement of Matrix metalloproteinases-9 in human adipocyte differentiation. *The Journal of Pharmacology and Experimental Therapeutics*, *312*, 1272–1279 No3 77263/1194036.

Buchanan, F. C., Fitzsimmons, C. J., Van Kessel, A. G., Thue, T. D., Winkelman-Sim, D. C., & Schmutz, S. M. (2002). Association of missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. *Genetics, Selection, Evolution*, *34*, 105–116.

Carmeli, E., Moas, M., Lennon, S., & Powers, S. K. (2005). High intensity exercise increases expression of matrix metalloproteinases in fast skeletal muscle fibres. *Experimental Physiology*, *90*, 613–619 15833756.

Caron, N. J., Asselin, I., Morel, G., & Tremblay, J. P. (1999). Increased myogenic potential and fusion of matrilysin-expressing myoblasts transplanted in mice. *Cell Transplantation*, *8*, 465–476.

Cauwe, B., & Opdenakker, G. (2010). Intracellular substrate cleavage: A novel dimension in the biochemistry, biology and pathology of matrix metalloproteinases. *Critical Reviews in Biochemistry and Molecular Biology*, *45*, 351–423.

Cha, M. C., & Purslow, P. P. (2010). Matrix metalloproteinases are less essential for the in-situ gelatinolytic activity in heart muscle than in skeletal muscle. *Comparative Biochemistry and Physiology — Part A: Molecular & Integrative Physiology*, *156*, 518–522.

Cha, M. C., & Purslow, P. P. (2011). Epinephrine induced MMP expression is different between fibroblasts and myoblasts. *Cell Biochemistry and Function*, *29*, 603–609.

Cha, M. C., & Purslow, P. P. (2012). Expressions of the matrix metalloproteinase and its inhibitor are modified by beta-adrenergic agonist ractopamine in skeletal fibroblasts and myoblasts. *Canadian Journal of Animal Science*, *92*(2), 159–166.

Chavey, C., Mari, B., Monthouel, M. N., Bonnafous, S., Anglard, P., Van Obberghen, E., & Tartare-Deckert, S. (2003). Matrix metalloproteinases are differentially expressed in adipose tissue during obesity and modulate adipocyte differentiation. *Journal of Biological Chemistry*, *278*(14), 11888–11896.

Chen, X., & Li, Y. (2009). Role of matrix metalloproteinases in skeletal muscle. *Cell Adhesion & Migration*, *3*(4), 337–341.

Clark, I. M., Swingler, T. E., Sampieri, C. L., & Edwards, D. R. (2008). The regulation of matrix metalloproteinases and their inhibitors. *The International Journal of Biochemistry & Cell Biology*, *40*, 1362–1378.

Coronato, S., Laguens, G., & Girolamo, V. (2012). Role of metalloproteinases and their inhibitors in tumors. *Medicina (B Aires)*, *72*(6), 495–502.

Du, M., Huang, Y., Das, A. K., Yang, Q., Duarte, M. S., Dodson, M. V., & Zhu, M. J. (2010). Meat Science and Muscle Biology Symposium: Manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. *Journal of Animal Science*, *91*, 1419–1427.

Du, M., Huang, Y., Das, A. K., Yang, Q., Duarte, M. S., Dodson, M. V., & Zhu, M. J. (2013). Manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. *Journal of Animal Science*, *91*, 1419–1427.

Du, M., Wang, B., Fu, X., Yang, Q., & Zhu, M. J. (2015). Fetal programming in meat production. *Meat Science*, *109*, 40–47.

Dunner, S., Sevane, N., Garcia, D., Cortes, O., Valentini, A., Williams, J. L., ... the GeMQual Consortium (2013a). Association of genes involved in carcass and meat quality traits in 15 European bovine breeds. *Livestock Science*, *154*, 34–44.

Dunner, S., Sevane, N., Garcia, D., Leveziel, H., Williams, J. L., Mangin, B., ... the GEMQual Consortium (2013b). Genes involved in muscle lipid composition in 15 european *Bos Turus* breeds. *Animal Genetics*, *44*, 493–501.

El Fahime, E., Torrente, Y., Caron, N. J., Bresolin, M. D., & Tremblay, J. P. (2000). In vivo migration of transplanted myoblasts requires matrix metalloproteinase activity. *Experimental Cell Research*, *258*, 279–287.

Garcia, L. G., Nicholson, K. L., Hoffman, T. W., Lawrence, T. E., Hale, D. S., Griffin, D. B., ... Smith, G. C. (2008). National beef quality audit-2005: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. *Journal of Animal Science*, *86*, 3533–3543.

Hadler-Olsen, E., Solli, A. I., Hafstad, A., Winberg, J., & Uhlin-Hansen, L. (2015). Intracellular MMP-2 activity in skeletal muscle in associated with type II fibers. *Journal of Cellular Physiology*(1), 160–169.

Heaton, M. P., Grosse, W. M., Kappes, S. M., Keele, J. W., Chitko-McKown, C. G., Cundiff, L. V., ... Laegreid, W. W. (2001). Estimation of DNA sequence diversity in bovine cytokine genes. *Mammalian Genome*, *12*, 32–37.

Hishikawa, K., Nakaki, T., & Frujii, T. (2000). Connective tissue growth factor induces apoptosis via caspase 3 in cultured human aortic smooth muscle cells. *European Journal of Pharmacology*, *392*, 19–22.

Huovila, A. P., Turner, A. J., Pelto-Huikko, M., Kärkkäinen, I., & Ortiz, R. M. (2005). Shedding light on ADAM metalloproteinases. *Trends in Biochemical Sciences*, *30*(7), 413–422.

Jorgenson, E., & Witte, J. S. (2006). A gene-centric approach to genome-wide association studies. *Nature Reviews Genetics*, *7*, 885–891.

Kelwick, R., Desanlis, I., Wheeler, G. N., & Edwards, D. R. (2015). The ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) family. *Genome Biology*, *16*(1), 113.

Kessenbrock, K., Plaks, V., & Werb, Z. (2010). Matrix metalloproteinases. *Regulators of the Tumor MicroenviromentNational institute of Health NIH Public Access*, *141*(1), 52–67.

Khokha, R., Murthy, A., & Weiss, A. (2013). Matrix metalloproteinases and their natural inhibitors in inflammation and immunity. *Nature Reviews Immunology*, *13*, 649–665.

Lee, S. H., Park, E. W., Cho, Y. M., Kim, S. K., Lee, J. H., Jeon, J. T., ... Yoon, D. (2007). Identification of differentially expressed genes related to intramuscular fat development in the early and late fattening stages of Hanwoo steers. *Journal of Biochemistry and Molecular Biology*, *40*, 757–764.

Lewis, M. P., Tippett, H. L., Sinanan, A. C., Morgan, M. J., & Hunt, N. P. (2000). Gelatinase-B (matrix metalloproteinase-9; MMP-9) secretion is involved in the migratory phase of human and murine muscle cell cultures. *Journal of Muscle Research and Cell Motility*, *21*, 223–233.

Lluri, G., & Jaworski, D. M. (2005). Regulation of TIMP-2, MT1-MMP & MMP-2 expression during C2C12 differentiation. *Muscle & Nerve*, *32*(4), 492–499.

Malemud, C. J. (2006). Matrix metalloproteinases (MMPs) in health and disease: An overview. *Frontiers in Bioscience*, *11*, 1696–1701.

Manello, F., & Gazzanelli, G. (2001). Tissue inhibitors of metalloproteinases and programmed cell death: Conundrums, controversies and potential implications. *Apoptosis*, *6*, 479–482.

Manello, F., & Medda, V. (2012). Nuclear localization of matrix metalloproteinases. Sciverse science Direct. *Progress in Histochemistry and Cytochemistry*, *47*, 27–58.
Manello, F., Tonti, G., & Papa, S. (2005). Matrix metalloproteinases inhibitors as anticancer therapeutics. *Current Cancer Drug Targets*, *5*, 285–298.
Martins, T. S., Sanglard, L. M. P., Silva, W., Chizzotti, M. L., Rennó, L. N., Serão, N. V. L., ... Duarte, M. S. (2015). Molecular factors underlying the deposition of intramuscular fat and collagen in skeletal muscle of Nellore and Angus cattle. *PloS One*, *10*(10), e0139943.
Matrisian, L. M. (1992). The matrix degrading metalloproteinases. *BioEssays*, *14*(7), 455–473.
McKenna, D. R., Roebert, D. L., Bates, P. K., Schmidt, T. B., Hale, D. S., Griffin, D. B., ... Smith, G. C. (2002). National beef quality audit-2000: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. *Journal of Animal Science*, *80*, 1212–1222.
Scollan, N. D., Dannenberger, D., Nuernberg, K., Richardson, R. I., MacKintosh, S., Hocquette, J. -F., & Moloney, A. P. (2014). Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science*, *97*(3), 384–394.
Miao, Z. G., Zhang, L. P., Fu, X., Yang, Q. Y., Zhu, M. J., Dodson, M. V., & Du, M. (2015). Invited review: Mesenchymal progenitor cells in intramuscular connective tissue development animal. *Animal*, *10*(01), 75–81 in press.
Murphy, G. (2010). Fell-muir lecture: Metalloproteinases: From demolition squad to master regulators. *International Journal of Experimental Pathology*, *91*, 303–313.
Nagase, H., Visse, R., & Murphy, G. (2006). Structure and function of matrix metalloproteinases and TIMPs. *Cardiovascular Research*, *69*, 562–573.
O'Hara, A., Lim, F. L., Mazzati, D. J., & Trayhurn, P. (2009). Microarray analysis identifies matrix metalloproteinases (MMPs) as key genes whose expression is up regulated in human adipose by macrophage-conditioned medium. *Pflügers Archiv - European Journal of Physiology*, *458*(6), 1103–1114.
Purslow, P. P. (2002). The structure and functional significance of variations in the connective tissue within muscleComp. *Biochem. Physiol. Part A*, *133*, 947–966.
Pages-McCaw, A., Ewald, A. J., & Werb, Z. (2007). Matrix metalloproteinases and the regulation of tissue remodelling. *Nature Reviews Molecular Cell Biology*, *8*(3), 221–233.
Purslow, P. P. (2005). Intramuscular connective tissue and its role in meat quality. *Meat Science*, *70*, 435–447.
Purslow, P. P., Archile-Contreras, A. C., & Cha, M. C. (2012). Manipulating meat tenderness by increasing the turnover of intramuscular connective tissue. *Journal of Animal Science*, *90*, 950–959.
Rosenblum, G., Meroueh, S., Toth, M., Fisher, J. F., Fridman, R., Mobashery, S., & Sagi, I. (2007a). Molecular structure and dynamics of the stepwise activation mechanism of a matrix metalloproteinases zymogen: Challenging the cysteine switch dogma. *Journal of the American Chemical Society*, *129*, 13566–13574.
Satyesh, K. S., Kamlesh, A., Hiroyasu, U., Santosh, N., Vivek, M., & Tripathi, B. R. (2014). Nuclear localization of catalytically active MMP-2 in endothelial cells and neurons. *American Journal of Translational Research*, *6*(2), 155–162www.ajtr.org /ISSN:1943-8141/AJTR1311011
Shiomi, T., Lamaitre, V., Darmiento, J., & Okada, Y. (2010). Matrix metalloproteinases, a disintegrin and metalloproteinases, and a disintegrin and metalloproteinases with thrompospodin motif in non-neoplastic disease. *National Institute of Health, NIH Public Access.*, *60*(7), 477–496.
Sung, M. M., Schulz, C. G., Wang, W., Sawicki, G., Bautista-Lopez, N. L., & Schulz, R. (2007b). Matrix metalloproteinase-2 degrades the cytoskeletal protein alpha-actinin in peroxynitrite mediated myocardial injury. *Journal of Molecular and Cellular Cardiology*, *43*, 429–436.
Tonti, G., Manello, F., Cacci, E., & Biagioni, S. (2009). Neural stem cells at the crossroads: MMPs may tell the way. *The International Journal of Developmental Biology*, *53*, 1–17.
Troeberg, L., & Nagase, H. (2007). Analysis of TIMP expression and activity. *Methods in Molecular Medicine*, *135*, 251–267.
Van Hul, M., Lupu, F., Dresselaers, T., Buyse, J., & Lijnen, H. R. (2012). Matrix metalloproteinase inhibitons affects adipose tissue mass in obese mice. *Clinical and Experimental Pharmacology and Physiology*, *39*, 544–550.
Vandenbrouke, R. E., & Libert, C. (2014). Is there new hope for therapeutic matrix metalloproteinase inhibition? *Nature Reviews Drug Discovery*, *13*, 904–927.
Verma, R. P., & Hansch, C. (2007). Matrix metalloproteinases (MMPs): Chemical–biological functions and (Q)SARs. *Science Direct. Bioorganic & medicinal Chemistry*, *15*, 2223–2268.
Williams, J. L., Dunner, S., Valentini, A., Mazza, R., Amarger, V., Checa, M. L., ... Leveziel, H. (2009). Discovery, characterization and validation of single nucleotide polymorphisms within 206 bovine genes that may be considered as candidate genes for beef production and quality. *Animal Genetics*, *40*, 486–491.
White, J. M. (2003). ADAMS: modulators of cell-cell and cell matrix interactions. *Current Opinion in Cell Biology*, *15*, 598–606.
Woessner, J. F. (1991). Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *The FASEB Journal*, *5*, 2145–2154.