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Review: Enhancing intramuscular fat development via targeting fibro-adipogenic progenitor cells in meat animals

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*In the livestock industry, subcutaneous and visceral fat pads are considered as wastes, while intramuscular fat or marbling fat is essential for improving flavor and palatability of meat. Thus, strategies for optimizing fat deposition are needed. Intramuscular adipocytes provide sites for lipid deposition and marbling formation. In the present article, we addressed the origin and markers of intramuscular adipocyte progenitors – fibro-adipogenic progenitors (**FAPs**), as well as the latest progresses in mechanisms regulating the proliferation and differentiation of intramuscular FAPs. Finally, by targeting intramuscular FAPs, possible nutritional manipulations to improve marbling fat deposition are discussed. Despite recent progresses, the properties and regulation of intramuscular FAPs in livestock remain poorly understood and deserve further investigation.*

Keywords: adipogenesis, fibro-adipogenic progenitors, skeletal muscle, marbling, meat

Implications

Intramuscular fibro-adipogenic progenitors reside in the interstitium of muscle fibers, and primary muscle bundles are the major source of intramuscular adipocytes where marbling fat deposits. Expanding intramuscular fibro-adipogenic progenitor pool and enhancing their commitment to adipogenic fate, in addition to their differentiation into lipid-laden adipocytes, provide attractive targets to facilitate marbling fat deposition thus improve palatability of meat.

Introduction

In farm animals, adipocytes are mainly clustered inside subcutaneous, intermuscular, visceral and mesenteric connective tissues, and some are scattered between and within muscle bundles. Fat pads located at subcutaneous, visceral and mesenteric depots have low commercial value and thus are generally considered as a waste for meat production. On the other hand, the quantity and distribution of intramuscular fat, or referred to as marbling fat, is highly desirable for enhancing meat flavor and palatability (Du *et al.*, 2013; Hausman *et al.*, 2014; Ngapo *et al.*, 2017 and 2018). Therefore, there are intensive efforts for increasing marbling fat deposition while reducing the overall fatness of animals.

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The contents of intramuscular fat (**IMF**) are mainly determined by the number and size of intramuscular adipocytes, of which the formation of intramuscular adipocytes is especially important because they provide sites for later marbling fat deposition. Most intramuscular adipocytes are deposited between primary and secondary muscle bundles in the perimysium of beef cattle (Harper and Pethick, 2004) and pigs (Chen *et al.*, 2019), while some marbling adipocytes can also be found within muscle bundles in the high-quality grade Japanese Black cattle (Hoshino *et al.*, 1990). There are also intramuscular adipocytes detected in humans (Agley *et al.*, 2013) and rodents (Bagchi *et al.*, 2018).

Recent studies show that adipocytes are derived from a pool of progenitor cells with dual potentials of adipogenic and fibrogenic differentiation, named fibro-adipogenic progenitors (**FAPs**) (Uezumi *et al.*, 2014). Here, we overviewed the origin and physiological behaviors of FAPs, factors controlling their proliferation and adipogenesis and potential strategies to enhance lipid accumulation in newly formed muscular adipocytes in order to increase marbling fat.

Molecular markers and heterogeneity of fibro-adipogenic progenitors

The origins of muscular fibro-adipogenic progenitors

The muscle stromal vascular fractions (**SVFs**), containing a mixture of mesenchymal stem/stromal cells (**MSCs**),

fibroblasts, immune cells and endothelial cells (Perruchot *et al*., 2013; Wosczyna *et al*., 2019), have been widely used for *in vitro* studies of intramuscular adipogenesis (Zhou *et al*., 2010; Jiang *et al*., 2013; Zhang *et al*., 2014; Sun *et al*., 2017 and 2018; Chen *et al*., 2019); however, the origins of intramuscular adipocytes had remained largely undefined until fairly recently.

In 2010, a subpopulation of non-myogenic mesenchymal stem cells, distinct from myogenic satellite cells, was identified to be the major source of intramuscular adipocytes in mice during muscle regeneration (Uezumi *et al*., 2010 and 2011) and later in humans (Uezumi *et al*., 2014), and these results are further confirmed by other independent studies (Agley *et al*., 2013; Arrighi *et al*., 2015). This subgroup of progenitor cells is platelet derived growth factor receptor α (also named CD140a)-positive (**PDGFRA**$^+$), and displays bipotency of differentiation into both lipid-laden adipocytes and collagen I-expressing fibroblasts, thus defined as FAPs (fibro/adipogenic progenitors). Bipotent PDGFRA$^+$ fibroblasts, unlike muscle satellite cells that locate beneath the basal lamina, are located in the interstitial space between muscle fibers and bundles of mice (Uezumi *et al*., 2010) and humans (Uezumi *et al*., 2014), where intramuscular fat accumulates (Duarte *et al*., 2013). Further studies showed that a large portion of intramuscular FAPs is generated from the embryonic Odd skipped-related 1 (**Osr1**)-positive fibroblasts (Vallecillo-García *et al*., 2017), and after birth, the levels of Osr1 in quiescent intramuscular FAPs become quite low (Stumm *et al*., 2018). New postnatal FAPs mainly arise from pre-existing muscle-resident PDGFRA$^+$ cells, not or rarely from PDGFRA$^-$ cells (Uezumi *et al*., 2011).

Bovine PDGFRA$^+$ cells within muscular SVFs are initially purified by our group (Huang *et al*., 2012a). PDGFRA$^+$ fibroblasts isolated from bovine skeletal muscles (Huang *et al*., 2012a; Guan *et al*., 2017) can be induced to differentiate into adipocytes by the traditional adipogenic induction cocktail, despite at low efficiency (Ma *et al*., 2018b). Besides, the abundance of PDGFRA in finishing Angus is correlated with its higher IMF contents when compared with Nellore (Martins *et al*., 2015). Thus PDGFRA is a marker of intramuscular adipocyte progenitors in beef cattle (Huang *et al*., 2012a; Miao *et al*., 2016). In pigs, PDGFRA$^+$ cells are located in the gaps of myofibers in *longissimus dorsi* muscle, and more PDGFRA$^+$ cells are detected in *longissimus dorsi* muscle of fat-type pigs compared with that of lean-type pigs at 180 days of age (Sun *et al*., 2017), albeit no difference was observed in the abundance of PDGFRA$^+$ cells or PDGFRA expression between *longissimus thoracis* (with higher IMF contents) and *semitendinosus* muscle (with lower IMF) in 180-day-old pigs (Chen *et al*., 2019).

Mouse muscular PDGFRA$^+$ cells also express other mesenchymal markers, such as mesenchymal intermediate filament, Vimentin, together with the well-discussed adipogenesis repressor, delta like non-canonical Notch ligand 1 (**Dlk1**, also known as preadipocyte factor 1 or **Pref1**) (Uezumi *et al*., 2010). Of note, Sca1 (Stem cells antigen 1)-positive cells overlap with over 85% of PDGFRA$^+$ cells in undamaged mouse muscle and up to 98% in injured muscles (Joe *et al*., 2010), and Sca1 is also commonly used to sort mouse FAPs (Fiore *et al*., 2016; Judson *et al*., 2017). However, a new study uncovered a subset of intramuscular Sca1$^+$/PDGFRA$^-$ cells within endothelial cells which derived from Myf5$^+$ progenitors in mice (Huang *et al*., 2014). The CD15 and PDGFRA label the similar subpopulation of cells within human skeletal muscle (Arrighi *et al*., 2015).

The heterogeneity of intramuscular fibro-adipogenic progenitors

Intramuscular FAPs, a subgroup of mesenchymal stromal cells (Dominici *et al*., 2006), constitute a heterogeneous pool of cells with divergent lineage dynamics. For instance, a sub-fraction of mouse intramuscular FAPs can differentiate as committed adipogenic progenitors, while the others express fibroblast markers even in the pro-adipogenic medium (Joe *et al*., 2010). More than 90% of FAPs derived from mouse muscle exhibit adipogenic potential, and the frequency reduces to ~60% in injured muscle (Uezumi *et al*., 2011), while in another study, the frequency is ~35% (Joe *et al*., 2010). The ratio of FAPs with adipogenic capacity is reported to be ~30% in unperturbed skeletal muscles of human (Uezumi *et al*., 2014). Accordingly, PDGFRA-sorted cells from bovine SVF display different *in vitro* adipogenic capabilities with distinct gene expression patterns (Huang *et al*., 2012a).

Furthermore, some, but not all of, FAPs are ciliated in mouse (Kopinke *et al*., 2017) and human skeletal muscles (Arrighi *et al*., 2017). Primary cilia are likely to mediate extracellular signals, such as transforming growth factor (**TGF**) β (Clement *et al*., 2013) and insulin-like growth factor (**IGF**) 1 (Dalbay *et al*., 2015) in human cell lines or Hedgehogs (**Hh**) in mouse (Kopinke *et al*., 2017), which regulate the lineage commitment and adipogenic differentiation of FAPs. The primary cilium shortens but maintains a signaling activity when human adipose and muscular FAPs morphs into fibroblasts *ex vivo* (Arrighi *et al*., 2017). On the other hand, the primary cilium of mouse muscular FAPs elongates at the early stage of adipogenic differentiation and then disappears in the mature adipocytes (Kopinke *et al*., 2017).

Moreover, PDGFRA$^+$ cells in mouse subcutaneous and visceral white adipose pads can be classified according to CD9 expression levels, and CD9^{low}/PDGFRA$^+$ progenitors can differentiate into adipocytes with dietary high-fat challenge, and CD9^{high}/PDGFRA$^+$ cells are fibrotic (Marcelin *et al*., 2017), supporting the heterogeneity of FAPs.

Proliferation of fibro-adipogenic progenitors

The importance of the proliferation of fibro-adipogenic progenitors on marbling

Interestingly, discernable intramuscular adipocytes in domestic animals appear later than adipocytes located in other fat

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depots (Du *et al*., 2013 and 2015). In beef cattle, adipocyte hyperplasia in visceral and subcutaneous fat greatly slows down after birth, but there are apparent cell hyperplasia observed in IMF depots in calves between 11 and 15 months of age (Cianzio *et al*., 1985). Of note, the later-formed intramuscular fat flecks tend to be smaller and rounder (Albrecht *et al*., 2006), thus benefiting marbling scores (Cheng *et al*., 2015). Consistently, in Asian cattle breeds with high marbling, intramuscular adipose tissue tends to be small and evenly distributed (Motoyama *et al*., 2016). Previous studies found that Wagyu FAPs are proliferated faster than Angus FAPs (May *et al*., 1994; Wei *et al*., 2015), which correlated with higher FAP density in Wagyu muscle (Duarte *et al*., 2013). Collectively, these studies suggest the higher proliferation capacity of FAPs contributes to the high marbling fat deposition (Albrecht *et al*., 2006; Kern *et al*., 2014; Wang *et al*., 2016; Kruk *et al*., 2018).

Factors regulating proliferation of intramuscular fibro-adipogenic progenitors

The mechanism underlying the proliferation of muscular FAP has been extensively studied using mice as models. As described earlier, FAPs acutely expand in injured muscles, and the activation of FAPs is necessary for muscle repair (Joe *et al*., 2010; Fiore *et al*., 2016). During muscle regeneration, activated FAPs obtain transient expression of Osr1. Odd skipped-related 1-positive FAPs either undergo apoptosis or return to the resident FAP pool after regeneration. These cells can also become adipocytes in fatty degeneration (Stumm *et al*., 2018). During muscle injury, intramuscular FAPs' accumulation is preceded by the appearance of inflammatory infiltration (Tidball, 2017), indicating that immune cells might regulate the activation of FAPs. Besides, age-related decline of dermal PDGFRA$^+$ is in parallel with the loss of immune cells (Zhang *et al*., 2019a). In chronic muscle damage, TGFβ1 (transforming factor β1) production in macrophages is elevated, which inhibits apoptosis (Lemos *et al*., 2015) and promotes proliferation of PDGFRA$^+$ cells in mouse muscle (Uezumi *et al*., 2011). In acutely damaged muscle, the expression of tumor necrosis factor α (**TNF**α) in macrophages is elevated, and TNFα directly induces apoptosis of FAPs (Lemos *et al*., 2015). Upon injection of cardiotoxin, interleukin (**IL**)-4 released by eosinophils enforces the proliferation of intramuscular FAPs (Heredia *et al*., 2013). Because inflammatory reaction is heavily dependent on tissue environment and the time elapsed since injury, it is difficult to clearly decipher the responses of FAPs to inflammatory cells.

Besides, growth factors such as platelet derived growth factor (**PDGF**) can provoke the proliferation of murine PDGFRA$^+$ FAP cells through activating PI3K (phosphatidylinositol 3- kinase) - AKT (protein kinase B) and MEK2 (mitogen activated protein kinase 2) signaling (Uezumi *et al*., 2014). Genetic knockout of vascular endothelial growth factor receptor 2 (**VEGFR2**) in mouse PDGFRA$^+$ cells blocks the pro-proliferation effects of retinoic acid on FAPs (Wang *et al*., 2017), indicating that vascular endothelial growth factor (**VEGF**) signaling promotes the proliferation of FAPs.

Until now, our knowledge on intramuscular FAP hyperplasia in livestock animals remains rudimentary. The SVF cells containing FAPs derived from Wagyu muscle possess higher proliferative ability than those from Angus (May *et al*., 1994; Wei *et al*., 2015). Also, SVF cells proliferate faster in fat-type Bamei than lean-type Landrace (Zhang *et al*., 2014). Certain nutrients, like conjugated linoleic acid (**CLA**) (Meadus *et al*., 2002) and vitamin A (Harris *et al*., 2018; Kruk et al., 2018), can augment marbling via increasing the number of intramuscular adipocyte precursors, although detailed mechanisms remain elusive.

Adipogenic differentiation of fibro-adipogenic progenitors

Regulation of the adipogenic differentiation of fibro-adipogenic progenitors

Fibro-adipogenic progenitors possess both fibrogenic and adipogenic potentials, and fibrogenesis of intramuscular FAPs has been depicted in our previous review (Miao *et al*., 2016). Because both adipocytes and fibroblasts are derived from FAPs, it had been postulated that enhancing adipogenic differentiation may correspondingly reduce fibrogenesis. Though this notion was supported by several studies (Huang *et al*., 2012a; Marcelin *et al*., 2017), enhanced adipogenesis and fibrogenesis are both detected in Wagyu skeletal muscles (Duarte *et al*., 2013), which could be due to the enhanced proliferation of FAPs resulting in the elevation of both fibrogenesis and adipogenesis. In agreement, enhanced adipogenesis and fibrogenesis were also observed in beef cattle offspring subjected to maternal overnutrition (Duarte *et al*., 2014). Besides, comparable fibrogenesis and collagen contents are found in Angus and Nellore cattle yet differing in IMF contents (Martins *et al*., 2015). These data suggest that enhanced intramuscular adipogenesis is not necessarily correlated with compromised fibrogenesis, and FAP proliferation has a major role in determining both processes.

The Zinc finger protein (**Zfp**) 423, a multi-zinc finger transcription factor, stands out as a key player in the commitment of progenitor cells to adipogenic lineage, which induces peroxisome proliferator activated receptor γ (**PPAR**γ) expression, which commits FAPs to preadipocytes, as well as converts preadipocytes to mature adipocytes (Gupta *et al*., 2012). Zfp423 is abundant in bovine SVF colonies with higher adipogenic capability, and enforced expression of Zfp423 propels adipogenic differentiation in low adipogenic cells and *vice versa* (Huang *et al*., 2012a). In agreement, bta-miR-23a blocks adipogenic genes expression and lipid accumulation in bovine intramuscular FAPs via directly targeting Zfp423 (Guan *et al*., 2017).

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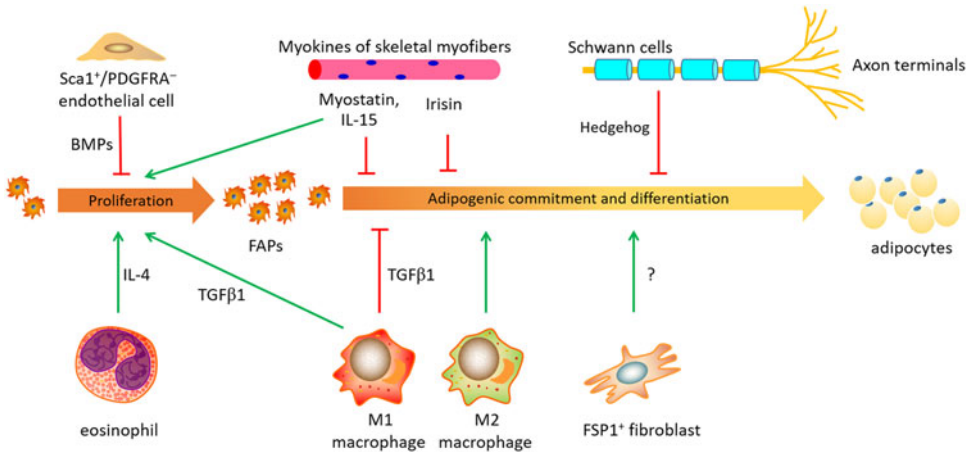

Figure 1 (Colour online) A schematic sketch of microenvironment niche affecting the proliferation and adipogenic differentiation of FAPs in mouse, human or pigs. FAPs, fibro-adipogenic progenitors; FSP1, fibroblast-specific protein-1; IL-15, interleukin 15; IL-4, interleukin 4; PDGFRA, platelet derived growth factor receptor alpha; TGFβ1, transforming growth factor β 1; Sca1+, Stem cells antigen 1 positive; BMP, bone morphogenetic protein.

Adipogenic differentiation of intramusclar compared to other fat depots

Generally, the lipid-storing capacity of intramuscular adipocytes is lower than subcutaneous adipocytes, consistent with the later development of intramuscular fat as compared to other fat depots. Indeed, the initial expression of genes related to lipogenesis and lipolysis in intramuscular preadipocytes is relatively slower when compared with subcutaneous preadipocytes (Wang *et al.*, 2013), and gene expression and/or activities of lipogenic and lipolytic enzymes are much lower in intramuscular fat than subcutaneous adipocytes (Jiang *et al.*, 2013). The sizes of adipocytes and lipid droplets are smaller in human intramuscular FAP-derived adipocytes than subcutaneous adipocytes (Arrighi *et al.*, 2015). Important similarities are identified in livestock animals. The diameter of bovine intramuscular adipocytes is smaller than those in subcutaneous adipose depots (Smith and Crouse, 1984). Likewise, intramuscular fat contains less lipid than subcutaneous adipose tissue in pigs (Kouba and Bonneau, 2009). The lower lipogenic capacity of intramuscular adipocytes is further confirmed by proteomic analysis (Gondret *et al.*, 2008), consistent with microarray analysis of gene expression in pigs (Zhou *et al.*, 2010). In alignment, the expression of leptin, an adipokine primarily secreted by lipid-laden adipocytes, is lower in intramuscular compared to subcutaneous fat (Gardan *et al.*, 2006). Recent RNA-seq study further confirmed the lower lipid metabolic capacity of intramuscular adipose tissue as compared to subcutaneous fat (Huang *et al.*, 2017).

The less maturity of intramuscular fat compared to other fat depots renders it less responsive to hormonal stimuli which alter lipid metabolism. Insulin-induced lipogenesis and catecholamine-induced lipolysis are lower in intramuscular fat compared with subcutaneous and perirenal adipocytes in growing pigs (Gardan *et al.*, 2006). Intramuscular SVFs isolated from semitendinosus muscles in neonatal pigs are less sensitive to glucocorticoids than that from subcutaneous adipose (Hausman and Poulos, 2004), correlated with their lower adipogenic activity (Chu *et al.*, 2017). The responses of intramuscular SVFs to other pro-adipogenic components, such as thiazolidinedione, also differ from subcutaneous counterparts (Poulos and Hausman, 2006).

The microenvironment of intramuscular fibro-adipogenic progenitors

The most significant difference between intramuscular FAPs and FAPs in other fat depots is the microenvironment. In an early study, purified intramuscular FAPs generate adipocytes only when transplanted to subcutaneous fat pads and glycerol-injected muscle, but not in healthy and intact muscle (Joe *et al.*, 2010). Besides, glycerol injection establishes a degenerative environment (more intramuscular adipocytes) and cardiotoxin injection generates a regenerative niche (less intramuscular adipocytes) (Mahdy *et al.*, 2015), and intramuscular FAPs reciprocally transplanted between degenerative and regenerative skeletal muscles can well adapt to different differentiation fates according to the new environment in mice (Uezumi *et al.*, 2010). These reports highlight the dominant regulatory effects of microenvironment on the differentiation from intramuscular FAPs into adipocytes. In addition to skeletal myofibers, infiltrating immune cells within skeletal muscles and others together build up a highly specialized niche environment for intramuscular FAPs (Figure 1).

The influence of skeletal myofibers and satellite cells

Early experiment in mice has shown that the adipogenesis of intramuscular FAPs can be strongly inhibited by the co-culture with myogenic cells (Uezumi *et al.*, 2010), indicating a cross-talk between intramuscular FAPs and myofibers. Muscle conditioned medium (**MCM**) was recently used to mimic the *in vivo* paracrine effects of skeletal muscle, and MCM can restrain the proliferation and differentiation of porcine subcutaneous preadipocytes (Han *et al.*, 2017).

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Myostatin is one of cytokines secreted from skeletal muscle, named as 'myokines'. Myostatin production in skeletal muscle is stimulated during chronic kidney disease in mouse model, and the increased myostatin promotes intramuscular FAP proliferation and conversion into fibrocytes (Dong *et al*., 2017). Myostatin can repress porcine glucocorticoid receptor expression in intramuscular adipocytes via elevating DNA methylation levels in its promoter (Chu *et al*., 2017). Myostatin also reduces glucocorticoid receptor at post-transcription level by upregulating expression of miR-124-3p in mice (Liu *et al*., 2019). Interleukin 15, another muscle-derived cytokine (Quinn, 2008), stimulates the proliferation of intramuscular FAPs, and impedes intramuscular FAP differentiating into adipocytes with upregulated Hedgehog signaling in mice (Kang *et al*., 2018). Besides, irisin, an exercise-induced myokine, reduces preadipocytes differentiation in humans (Huh *et al*., 2014).

The content of IMF varies among different muscles in the same animal (Sharma *et al*., 1987; Font-i-Furnols *et al*., 2019, likely due to the difference in muscle fiber composition and locomotion (Picard *et al*., 2018). The content of IMF is higher in the belly and lower in the ham (Kouba and Bonneau, 2009). For pigs, the most cranial part of loin presents the highest IMF content, as well as the *Biceps femoris* muscle of ham (Font-i-Furnols *et al*., 2019). As mentioned earlier, SVFs, which contain FAPs, derived from *longissimus thoracis* muscle present earlier and greater lipid accumulation than those from *semitendinosus* muscle, which is consistent with the higher IMF content in *longissimus thoracis* (Chen *et al*., 2019).

Besides the regulatory effects of myofibers on the FAPs, it was recently reported that satellite cells are able to inhibit fibrogenesis of FAPs through secreting exosomes containing miR-206, a process believed to be critical for suppressing fibrogenesis induced by skeletal muscle hypertrophy (Fry *et al*., 2017).

The influence of immune cells

Immune cells also regulate the fates of intramuscular FAPs. Fibro-adipogenic progenitors and macrophages (CD68 positive) are located in very close proximity in human degenerating skeletal muscles (Moratal *et al*., 2018), suggesting a potential interaction between intramuscular FAPs and macrophages *in vivo*.

Macrophages are schematically classified into 'proinflammatory' M1 and 'anti-inflammatory' M2 subgroups (Murray *et al*., 2014). 'Proinflammatory' M1 macrophages are recruited at the initial stage of muscle regeneration, and 'anti-inflammatory' M2 macrophages are subsequently activated in a later regeneration phase (Chazaud, 2016), although a new study found that both M1 and M2 macrophages are broadly activated at the early stage of acute skeletal muscle injury (Wang *et al*., 2018). The transition from a pro- to anti-inflammatory status in the niche profoundly affects intramuscular adipogenesis in mice (Dammone *et al*., 2018). Indirect co-culture with conditioned media showed that cytokines secreted by IL-1β-polarized macrophages drastically reduce intramuscular FAP adipogenic potential via stimulating SMAD2 (SMAD family member 2) signaling, and factors released by IL-4-polarized macrophages conversely enhance cellular lipid accumulation and expression of adipogenic markers, thus facilitating intramuscular FAPs adipogenesis (Moratal *et al*., 2018).

Specifically, TGFβ1, secreted from macrophages during the regeneration phase in damaged muscle, is the most critical profibrogenic cytokine via stimulating the receptor-SMAD cascades (Miao *et al*., 2016). Transforming growth factor β1 represses adipogenic differentiation of FAPs both *in vivo* and *ex vivo* (Lee, 2018; Zhang *et al*., 2019a). Knockdown of TGFβ1 receptor accelerates adipogenic differentiation in porcine preadipocytes (Zhang *et al*., 2019b) and murine 3T3-L3 cells (Zhang *et al*., 2015). Furthermore, the inhibitory effects of TGFβ1 on intramuscular adipogenesis are time-dependent. Co-injection of TGFβ1 with glycerol presents greater repressive effects than its administration 4 days following glycerol injection (Mahdy *et al*., 2017). In aggregate, the differentiation fates of intramuscular FAPs are regulated by the intensive interaction with the surrounding macrophages and other immune cells.

However, studies discussed earlier were mainly conducted under pathological conditions, and there is currently lack of evidence about the contribution of immune cells in FAP differentiation and intramuscular marbling development in domestic animals under physiological conditions. A recent study discovers that genes related with T-cell activation are differentially expressed in porcine muscles divergent in feed efficiency and product quality (Horodyska *et al*., 2018), indicating a possible interaction between immune cells and myofibers in skeletal muscle, likely with intramuscular FAPs under homeostatic conditions in farm animals.

The influence of fibroblasts and other cells

A latest study on mouse white fat suggests the critical contribution of fibroblasts to adipogenesis (Zhang *et al*., 2018). Fibroblast-specific protein-1 (**FSP1**) is a marker for resident fibroblasts in some tissues including skeletal muscle. Fibroblast-specific protein-1$^+$ fibroblasts in WAT are adjacent to the preadipocytes but devoid of adipogenic potential. Ablation of FSP1$^+$ fibroblasts results in loss of adiposity, arguing that the FSP1$^+$ fibroblasts function in providing an essential adipogenic niche for FAPs (Zhang *et al*., 2018). In this regard, it is intriguing to speculate the interaction between non-adipogenic fibroblasts and intramuscular adipogenesis in livestock animals.

Another recent study described the inhibitory role of Sca-1$^+$/CD31$^+$/PDGFRA$^-$ myo-endothelial progenitors on the adipogenic differentiation of FAPs (Huang *et al*., 2014). Myo-endothelial progenitors are a group of newly identified subset of endothelial cells developmentally derived from Myf5 lineage and are located in the inter-myofiber spaces. Huang and colleagues identified that myo-endothelial progenitors inhibit intramuscular adipogenesis through bone morphogenetic protein (**BMP**) signaling. Deletion of BMP receptor 1a (**Bmpr1a**) in Myf5$^+$ cells abolished the inhibitory

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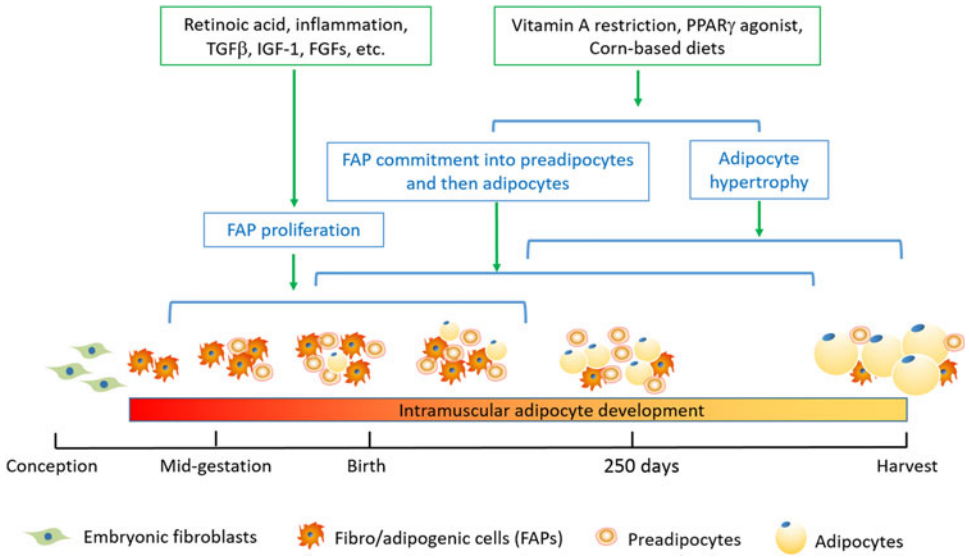

Figure 2 (Colour online) Timeline for nutritional strategies to enhance intramuscular FAPs and their differentiation into adipocytes in beef cattle. The stages including FAP proliferation, FAP commitment into preadipocytes and then adipocytes, and adipocyte hypertrophy are not mutually exclusive; instead, these processes represent progressive changes. Because of the conservativeness of adipose tissue development, it should be applicable to other species. However, due to the difference in maturity of animals at birth and at harvest, the timeline needs to be adjusted accordingly. FAPs, fibro-adipogenic progenitors; FGFs, fibroblast growth factors; IGF-1, insulin-like growth factor 1; PPARγ, peroxisome proliferator activated receptor γ; TGFβ, transforming growth factor β.

effect of myo-endothelial progenitors on the adipogenic differentiation of FAPs, resulting in enhanced intramuscular adipogenesis in mice (Huang *et al.*, 2014).

Hedgehog signaling exerts a conserved and inhibitory role on fat formation (Suh *et al.*, 2006). In skeletal muscle, Hedgehog signals (e.g. Desert Hedgehog, **Dhh**) are primarily produced by Schwann cells. Desert Hedgehog expression in Schwann cells is induced by cardiotoxin injection, and the extracellular Dhh signal can transduce into intramuscular FAPs via the primary cilia on their cell surfaces and block adipogenesis of intramuscular FAPs (Kopinke *et al.*, 2017).

Nutritional strategies to improve marbling via targeting intramuscular fibro-adipogenic progenitors

As reviewed previously (Estany *et al.*, 2017; Park *et al.*, 2018), genetic background or breeds, management (e.g. early weaning, castration and prolonged feeding) can effectively increase IMF percentage and marbling score. Here, we only focus on nutritional manipulations targeting intramuscular FAPs (Figure 2).

Prenatal nutrition

The fetal and neonatal stages are most effective in promoting FAP proliferation and intramuscular adipocyte formation (Du *et al.*, 2013 and 2015). Through binding to retinoic acid receptors, retinoid acids are required for adipogenesis. We recently found that maternal supplement of vitamin A or retinoid acid expands FAP population in mice (Wang *et al.*, 2017), and injection of vitamin A at birth and 1 month of age promotes intramuscular fat development in Angus beef cattle (Harris *et al.*, 2018). Because vitamin A deficiency in beef cattle occurs during the dry season when β-carotenoid content in forage becomes very limited, vitamin A supplementation at fetal and newborn stage provides a feasible strategy to increase intramuscular adipogenesis and marbling fat development in beef cattle (Kruk *et al.*, 2018).

It is widely accepted that maternal nutrition affects adipose tissue and skeletal muscle development in lamb (Zhu *et al.*, 2006), beef cattle (Robinson *et al.*, 2013) and pigs (Oksbjerg *et al.*, 2013). Maternal nutrient deficiency leads to overall increase in offspring fatness when fed with a high-energy diet (Dandrea *et al.*, 2001; Zhu *et al.*, 2006; Symonds *et al.*, 2012), likely due to the adipocyte hypertrophy. On the other hand, maternal over-nutrition promotes intramuscular adipogenesis. Overfeeding ewes leads to a higher density of intramuscular adipocytes in fetal (Yan *et al.*, 2010) and adult lambs (Yan *et al.*, 2011), accompanied with elevated collagen accumulation (Huang *et al.*, 2012b), indicating that maternal over-nutrition might increase the number of intramuscular FAPs.

Postnatal nutrition

Intramuscular adipogenesis occurs at a later stage compared to other fat depots (Albrecht *et al.*, 2015). Postnatal hyperplasia of intramuscular preadipocytes also plays an important role in marbling formation (Albrecht *et al.*, 2006), and individuals with a high capacity to create more preadipocytes within muscle are recommended in cattle breeding (Harper and Pethick, 2004). Supplementation of CLA during the fattening stage increases IMF accumulation while decreases subcutaneous deposition in pigs (Wiegand *et al.*, 2002) and cattle (Zhang *et al.*, 2016). Possible interpretation is that CLAs promote the development of preadipocytes in intramuscular SVF cells, which contain FAPs (Meadus *et al.*, 2002), while limiting the adipogenic differentiation of subcutaneous SVF cells (Zhou *et al.*, 2007). Vitamin A restriction for

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10 months during the finishing state of steers greatly increased IMF, possibly through enhanced proliferation of intramuscular preadipocytes, expanding the pool of intramuscular adipocytes; such effect was not observed in other fat in subcutaneous depots likely due to its inability for adipocyte expansion (Kruk *et al.*, 2018).

Other promising strategies

Peroxisome proliferator activated receptor γ (PPARγ), a ligand-dependent transcription factor, is a master regulator of adipogenesis, and its agonists (rosiglitazone, thiazolidinedione, pioglitazone and others) are generally effective in treating metabolic dysfunction and diabetes (Ma *et al.*, 2018a). Excitingly, PPARγ agonists promote IMF deposition while improving insulin sensitivity in type 2 diabetes (Mayerson *et al.*, 2002). Consistent results are observed in mice (Muurling *et al.*, 2003) and rats (Lessard *et al.*, 2004). Similarly, dietary supplementation of thiazolidinedione (Chen *et al.*, 2013) or pioglitazone hydrochloride (Jin *et al.*, 2018) noticeably promotes IMF accumulation in finishing pigs, without affecting backfat thickness. Further work showed that the activation of PPARγ specially enhance adipogenesis of porcine muscular SVFs (Li, 2018), likely due to the enrichment of FAPs in intramuscular fat compared to other fat pads. Thus, it is promising to seek chemicals or supplements as PPARγ agonists to specifically increase marbling.

Previous works propose that intramuscular adipocytes prefer glucose for *de novo* fatty acid synthesis (Smith and Crouse, 1984; Rhoades *et al.*, 2007; Wang *et al.*, 2013); thus dietary glucose can be used as a lipogenic substrate. Compared with the hay-based diet, corn-based diet can increase glucose uptake in intramuscular adipocytes, thus increasing IMF contents in America Wagyu (Chung *et al.*, 2007) and Barrosã bulls, but not Alentejana bulls under the same condition (Costa *et al.*, 2013). Conversely, hay-based diet in the finishing stage generates lower marbling in Jersey steers (Arnett *et al.*, 2012).

Conclusion

Skeletal muscular PDGFRA^{+} FAPs are the major sources of intramuscular adipocytes, which provide a key target to promote intramuscular adipocyte development and marbling fat deposition. Consistently, the proliferative and adipogenic potential of muscular FAPs correlates with marbling fat deposition, and the density of FAPs differs among breeds with different IMF contents and marbling scores. Growing efforts focusing on expanding intramuscular FAP pool, their commitment to preadipocytes and final differentiation into mature adipocytes, through nutritional and pharmaceutical manipulations, have yielded promising results. Moreover, as progenitor cells with dual potency of adipogenesis and fibrogenesis, the differentiation fate of intramuscular FAPs is largely dependent on its niche environment, which warrants further investigation in order to enhance a pro-adipogenic niche.

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Declaration of interest

All authors declare no conflicts of interest.

Ethics statement

None.

Software and data repository resources

No new software or database was generated as part of the outcomes of this work.

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