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Review

Actin associated proteins function as androgen receptor coregulators: An implication of androgen receptor's roles in skeletal muscle

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ABSTRACT

This review of androgen receptor (AR) coregulators, which also function as actin-binding proteins, intends to establish the connection between actin cytoskeletal components and androgen signaling, especially in skeletal muscle. In cellular and animal models, androgen activated AR modulates myoblasts proliferation, promotes sexual dimorphic muscle development, and alters muscle fiber type. In the clinical setting, administration of anabolic androgens can decrease cachexia and speed wound healing. During myogenesis and regeneration of skeletal muscle in embryo and adult, the membrane of myoblasts fuse and the actin cytoskeleton is rearranged to form an alignment with myosin to form myotubes then ultimately the myofibrils. Contraction of skeletal muscle promotes the growth of myocytes by coordinating signals from the neuromuscular junction to intra-myofibrils through costameres, the functional structure comprised of signal proteins closely associated with actin filaments and involved in muscular dystrophy. Therefore, the discovery of actin-binding proteins functioning as AR coregulators implies that androgen signaling is tightly regulated during the process of the development and regeneration of skeletal muscle. The search for selective androgen receptor modulators (SARM) that act precisely in skeletal muscle instead of other tissues could target the engineering of a SARM–AR complex that selectively recruits these coregulators.

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1. Introduction

Skeletal muscle has been known to be one of the tissues targeted by androgens for decades. It is generally believed that the masculine muscle mass developed during puberty in men is contributed in majority by androgen. Testosterone replacement in hypogonadal men indeed improves muscle mass and strength. Also, androgenic steroids have been widely used by athletes to augment muscle size and strength. This effect of androgen in

skeletal muscle can be very useful in treating muscle-wasting conditions in the aging population, as well as in patients suffering severe burn injury, undergoing chemotherapy, and with AIDS. The supplementation of androgen in muscle increases protein synthesis, RNA polymerase activity, the uptake of 2-deoxyglucose, and enhances glycogen synthesis. Therefore, androgens are also called anabolic steroids. The adverse effect of applying anabolic androgenic steroid supplementation for rebuilding muscle mass is the risk of developing prostate diseases in men, virilization in women, and changing lipid profiles which might increase the risk of cardiovascular disease. Recently, several promising androgen analogues were developed, as potential selective androgen receptor modulators (SARMs), which claim to preferentially act on skeletal muscle but not the prostate [1–3]. These SARMs were developed from

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non-steroidal androgens to be given orally. They bind to the androgen receptor (AR) with high affinity and exert strong pharmacological activity in selective tissues. However, the mechanism for this selectivity is not well understood.

Androgen action is mediated through the AR which belongs to the nuclear receptor (NR) superfamily and functions as a transcriptional regulator in response to cognate ligands [4]. Like other members of this superfamily, the AR consists of a conserved DNA-binding domain (DBD), a ligand-binding domain (LBD), and a less conserved amino-terminal domain (N-terminus) [5]. After ligand binding, the AR dissociates from chaperone proteins, forms dimers, translocates into the nucleus, recognizes its response element on the promoter of target genes, and recruits transcriptional factors to turn on transcription [6]. NRs have two separate activation functions (AFs) mediating transactivation in the N-terminus (AF-1) and the LBD (AF-2) [7]. The activity of AF-1 is ligand-independent while ligand-binding regulates AF-2 activity. However, AF-2 in the AR is relatively weak and instead of interacting with coregulators like other steroid receptors (SR), it interacts strongly with the AR N-terminus [8]. The interaction of the N-terminus and AF-2 has been demonstrated to influence the protein stability of the AR and ligand dissociation from the AR [9].

In addition to ligand binding, AR function is regulated by other mechanisms. First, the expression of the AR and its regulation influence the overall activity of the AR in some tissues under particular conditions. The relative abundance of the AR in descending order is the adrenal gland, followed by the epididymis, prostate, skeletal muscle, kidney, liver, and heart. AR expression is upregulated by androgen, follicle-stimulating hormone, prolactin, and epidermal growth factor and downregulated by *trans*-retinoic acid and androgens in some cell lines [10]. Second, post-translational modifications of the AR, including acetylation, phosphorylation, ubiquitination, and sumoylation, have been reported to affect the activity of the AR [11–14]. Sumoylation of the AR results in decreased activity in the presence of ligands, while phosphorylation enhances AR activity independent of ligands. The mechanism of decreased AR activity following sumoylation is not clear. On the other hand, phosphorylation may increase the affinity of the AR for coregulators, resulting in enhanced transactivation. Finally, the discovery of coregulators broadened the scope of AR functional regulation. Since 1995, many NR associated proteins have been identified by yeast two-hybrid or affinity column systems. Further characterization of these proteins demonstrated their ability to regulate the transactivation of their associated receptors. These proteins either repress unliganded receptors or enhance liganded receptors and are categorized as members of the coregulator family. Coregulators interact with either liganded or unliganded receptors and modulate receptor activity by multiple mechanisms. They may remodel chromatin by their intrinsic histone acetylation (HAT), or deacetylation (HDAC) activity, or recruit proteins bearing such activities, to regulate transcription of target genes [15]. In addition to this mechanism, coregulators may facilitate receptor nuclear trafficking, promote receptor DNA binding, recruit or function as basal transcription factors (TF), or modify receptors as described in previous publications [16–19].

The various abundances of coregulators among tissues contribute to the tissue specificity of SERMs [20]. In an attempt to identify AR coregulator(s) in skeletal muscle, we screened cDNA library from skeletal muscle and found two actin-binding proteins, supervillin and gelsolin, were constantly fished out in the yeast two-hybrid system. Cytoskeleton reorganization is required for myoblast fusion to form myotubes during muscle development and regeneration. This observation led us to hypothesize that the cytoskeleton rearrangement couples with androgen signaling in skeletal muscle. Further study demonstrated that overexpression of

monomeric actin enhanced AR transactivity, while overexpression of Rac-induced actin polymerization and reduced AR transactivity [21].

In this review, we summarize the androgen response in skeletal muscle, then focus on AR coregulators functioning in cytoskeleton rearrangements to overview their mechanism in modulating AR transactivity, their biological function in skeletal muscle, and discuss their potential roles in coupling AR signaling to skeletal muscle function.

2. Biological function of androgen in skeletal muscle

The sexual dimorphic skeletal muscle mass is believed to be regulated by the male sex hormone, androgen, which surges in secretion during puberty. The direct evidence for this primarily emerged from animal models. In rodent, the levator ani and the bulbocavernosus muscle located at the pelvic floor and lower urinary tract are well known sexually dimorphic muscles that appear to be highly sensitive to androgens [22,23]. It has been demonstrated that androgen administration results in muscle hypertrophy rather than altering the number of myofibrils [24]. However, androgen treatment of the frog laryngeal muscle, a sexual dimorphic skeletal muscle, produces increased muscle fibers during the metamorphosis stage, but not in the adult frog [25,26]. Therefore, the androgen effects in skeletal muscle might be muscle type and developmental stage specific, which may result from AR susceptibility, cell context, and the environment.

Several studies utilized satellite cells, the major muscle source after maturity, to demonstrate the androgen effect. Notably, two studies reported that testosterone induces satellite cells proliferation in rat levator ani muscle [27,28]. However, this effect seems to be partially reproduced *in vitro* according to other studies utilizing satellite cell culture. One report demonstrated that androgen had no effect on actin-accumulation of satellite cell-derived myotube culture [29]. In isolated and cultured satellite cells from skeletal muscle, AR expression can be induced by androgen treatment [30,31]. However, testosterone had no effect on satellite cell proliferation and decreased its differentiation [30]. Indirectly, androgen can also regulate motor neuron innervation in these sexual dimorphic muscles to affect their growth [32]. Lack of neuron innervation in the cell culture system might contribute to the partial response to androgen observed.

In addition to animal and primary culture studies, the myoblast cell line culture has also been applied. However, few successful cases have been reported using cell culture to demonstrate androgen function in skeletal muscle. It is probably due to the low or non-detectable level of AR in these myoblast cell lines. One report in 1975 showed the first evidence for a direct response of myoblasts to androgens by increasing DNA labeling index and decreasing the time spent in the G1 phase of the cell cycle [33]. In order to analyze the role of androgen-AR signaling pathway in skeletal muscle development, we established AR stably overexpressed myoblast C2C12 cell line by stable transfection of the plasmid expressing Flag-tagged AR. The results indicate that the androgen-AR signaling pathway may suppress skeletal myoblast cell growth and accelerate myoblast cell differentiation. Furthermore, we demonstrated that AR regulates myoblast differentiation via enhanced myogenin expression [34]. In order to eliminate the possible artificial effect of constitutive AR overexpression on myoblast cells, we established another expression plasmid, pIRESNP-fAR, which expresses AR under the control of upstream regulatory region of AR gene. Our recent study demonstrated the upregulation of AR proteins expression during differentiation of C2C12 myoblast cells containing the transgene of pIRESNP-fAR. Analysis of quadriceps skeletal muscle of ARKO mice indicates an increase of fast-twitch

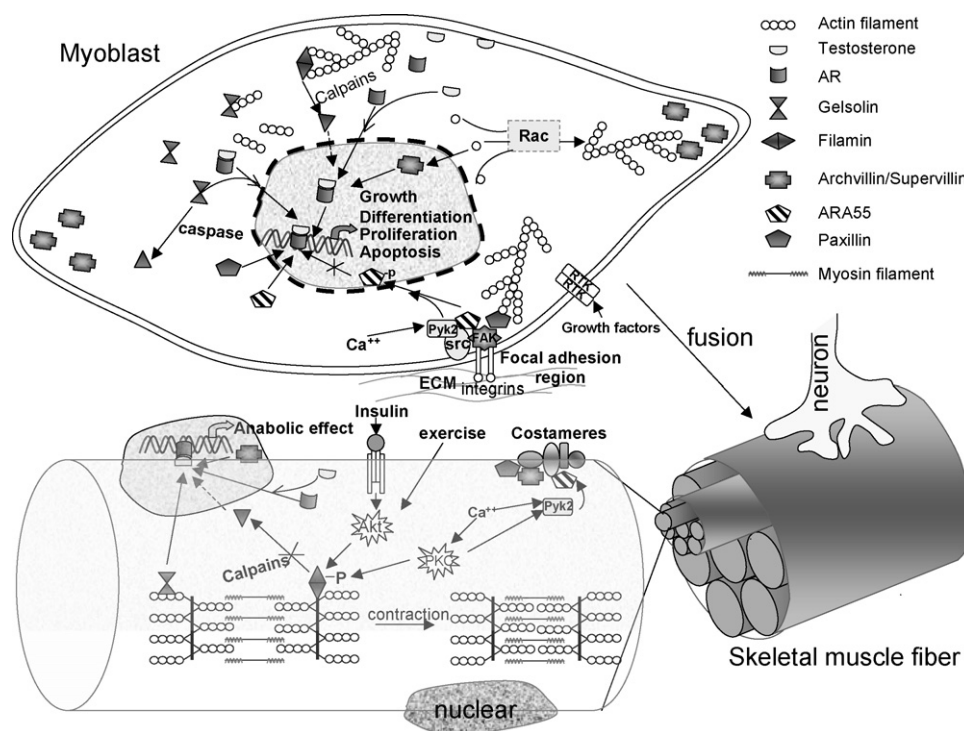


Fig. 1. Diagram of AR action in the presence of actin associated proteins. The location and action of actin associated protein in conducting signaling, cytoskeleton reorganization, and their interaction with AR in myoblast and mature myofibril of skeletal muscle are depicted.

muscle fiber type cells compared to wild type mice [35]. Androgens also stimulate the differentiation of pluripotent mesenchymal cells to the myogenic lineage, which suggests progenitor cells participate in the response to androgen by increasing muscle mass [36].

3. Anabolic androgen

Anabolic steroids represent a family of synthetic androgens with tissue-selective activity. This class of medications promotes skeletal growth, increases mass and strength of skeletal muscle, and leads to nitrogen retention while having reduced virilizing and behavioral effects as compared to testosterone. Administration of anabolic androgen to increase lean body mass and muscle strength has been observed in athletes. Anabolic steroids are also used increasingly to preserve or restore skeletal muscle mass and promote wound healing [37]. The anabolic steroid oxandrolone has been one of the most widely studied agents. Oxandrolone has been reported to reverse the cachexia associated with HIV infection, and decrease cachexia and speed wound healing in those with severe burns [38].

The anabolic activity of anabolic androgens is evaluated by the nitrogen balance (protein anabolic activity) and the growth weight of the kidney (renotrophic activity). However, these are indicators of general anabolic activity, not specific for skeletal muscle. The method of determining myotrophic activity by growth weight, in muscle levator ani (m.l.a.) of rats, has been practiced on a large scale. When evaluating the potency of a newly synthesized anabolic androgen, the expression of anabolic activity as the ratio of studied and standard steroid doses causing organ weight doubling in comparison with the control, or minimum doses causing growth in m.l.a. weight by 50% are usually used. Another way to evaluate the properties of anabolic androgen is the anabolic index, which is characterized by the ratio of myotrophic activity to androgenic activity. This index indicates a steroid to be anabolic if the number is greater than one while an androgenic steroid if less than one [37].

Anabolic androgen action observed includes stimulation of the activity of nuclear RNA polymerases I and II, increase of RNA syn-

thesis, and induction of protein synthesis resulting in increase of enzyme activity. Increasing expression of IGF-1 in satellite cells and suppressing catabolic effect of glucocorticoid receptor are mechanisms for anabolic androgen to stimulate muscle satellite cell proliferation and augment anabolism [39,40].

The abuse of anabolic androgens, however, should be taken into concern due to the well known adverse effect including disturbing regular androgen production, acne, aggressive behavior, virilization, and potentially increasing risk of cardiovascular disease through decreasing HDL production [41]. Therefore, the development of SARMs to reduce the adverse effect is highly demanded. AR coregulators that specifically modulate AR function in skeletal muscle are therefore valuable for developing SARMs for this purpose.

4. Actin-binding proteins function as AR coregulators in skeletal muscle

During skeletal muscle myogenesis, myoblasts proliferate, differentiate, and fuse to form myotubes that contain multi-nuclei with striated myofibrils. This process involves reorganization and elongation of actin filaments, which is a dynamic and complex process. Several proteins initially characterized as actin-filament structural proteins or involved in actin-filament forming complexes have been found to be AR coregulators (Fig. 1).

1. Gelsolin is a potent actin filament severing and capping protein. The severing process is initiated by binding to the actin filament then breaking the actin filament in half. After severing, gelsolin attaches to the barb-end to prevent polymerization of actin filament. Once gelsolin is uncapped from the filament, polymerization occurs and the cytoskeleton is reorganized and remodeled. The N-terminus of gelsolin is sensitive to phosphatidylinositol bisphosphate (PIP₂) that controls the uncapping of gelsolin, and the C-terminus of gelsolin is sensitive to Ca²⁺,

which activates the actin binding and severing. The C-terminus is also the regulation domain that controls the severing activity of the N-terminus [42]. The linkage between the N- and C-terminus of gelsolin is cleaved by caspase-3 [43]. Once cleaved, the severing activity of the N-terminus is not controlled by Ca^{2+} and therefore break-down of the cytoskeleton destructs cell integrity that usually accompanies the apoptosis process.

Gelsolin is identified as a coactivator of AR that interacts with AR in an androgen-dependent manner and enhances AR transactivation. The mechanism through which gelsolin enhances AR transcription involves colocalization with AR during nuclear translocation. The interaction motif ³⁸⁹FKQFF³⁹³ located in the C-terminus of gelsolin mediates gelsolin's interaction with AR DNA-binding and ligand-binding domains [44 and unpublished data]. During myogenesis, gelsolin expression in myoblasts steadily increases from initial stages of differentiation then decreases in terminally differentiated myofibrils. The interaction between gelsolin and myofibril filaments also increases in differentiated myotubes indicating the importance of gelsolin in the development of skeletal muscles [45]. The variation in the amount and myofibril association of gelsolin during the maturation of myotubes implies that AR transactivity could also fluctuate and therefore regulates the process.

- Supervillin is a 205 kDa actin-binding protein with structural homology to gelsolin and villin. It was first cloned from the actin filaments complex in bovine neutrophil plasma membrane and later found to be highly expressed in muscle [46]. The C-terminus of supervillin is gelsolin-like and the N-terminus interacts with actin. Supervillin contains a functional nuclear localization signal located in the middle of the protein that contributes to its nuclear distribution under low density cell culture. Its muscle specific isoform, archvillin, was later cloned and characterized to associate with dystrophin of costameres and also to localize within the nucleus in myoblasts. The importance of archvillin in early myogenesis is suggested by the observations of its increasing expression during myogenesis, its polarizing localization in differentiating myotubes and dominant-negative inhibition effect of archvillin fragments in myotubes formation [47].

Both supervillin and archvillin contain the domain that is required for interacting with AR and enhancing AR transactivity [47,48]. Both the N-terminus and C-terminus of AR were found to interact with supervillin. The truncated fragment of supervillin containing the functional nuclear localization signal and AR interaction domain is sufficient to enhance AR transactivity and is relatively more potent than full-length supervillin. This implicates there is a regulatory domain controlling the coactivator function of supervillin. We also demonstrated the dynamics of actin polymerization status in cells modulate the coactivator function of supervillin toward AR, which is decreased when increasing actin polymerization by constitutive Rac and enhanced by overexpressing actin monomer [21]. Archvillin, the skeletal muscle specific isoform, localizes in both the nucleus and cytoplasm of un-differentiated myoblast cells, then increases expression in cytoplasm and membrane of differentiated myotubes. The N-terminus fragment of archvillin exhibits a dominant-negative function in myogenesis [47]. It is therefore predicted that archvillin enhances AR transactivity during early myogenesis and the dominant-negative fragment of archvillin impedes myogenesis through interfering with AR function.

- The 280 kDa f-actin cross-linking protein, filamin A (FLNa), first identified from yeast two-hybrid screening interacts with the AR in a ligand-enhanced manner. AR is unable to translocate to the nucleus and activate transcription in response to androgen in the filamin-negative M2 cell line [17]. Reconstitution of filamin in M2 cells enables AR to translocate and activate a reporter gene

upon androgen treatment demonstrating that this cell line lacks filamin, but not other factors, for AR transcription. The filamin fragment that interacts with AR also inhibits AR transcriptional activity. Further investigation found that a naturally occurring FLNa fragment generated by calpain digestion is 110 kDa, and can colocalize with AR into the nucleus. This FLNa fragment interacts with the AR hinge domain to interfere with AR inter-domain interactions and coactivator recruitment hence inhibits AR transactivity [49].

There are three isoforms of FLN, named A (1 or alpha), B (beta), and C (2 or gamma). FLNa (FLN1, alpha-FLN) expresses ubiquitously while FLNc (FLN2, gamma-FLN) expresses highly in striated muscles [50]. Both of them can interact with AR [17]. The expression of FLNc is dynamically regulated in the myogenic progenitor during muscle development and regeneration. This expression pattern of FLNc is consistent with the observation in human skeletal muscle culture and C2C12 cell line where FLNc is absent in proliferation, but upregulated during differentiation. In addition, FLNc localises in Z-discs already at the first stages of Z-disc formation, suggesting its role in Z-disc assembly [51,52]. Besides expression, posttranslational modifications of FLN by Akt and PKCalpha have been reported. Insulin and exercise both activate Akt, which phosphorylates FLN, although endurance exercise increases FLNa phosphorylation only, but not FLNc, in skeletal muscle [53]. This isoform-restricted modification is also observed in PKCalpha signaling which results in phosphorylation of FLNa and FLNc but not FLNb [54]. Phosphorylation of FLNc by PKCalpha decreases its interaction with calpain 1 in muscle that results in increasing myotubes adhesion [55]. Calpains, the calcium-dependent cysteine-protease family, cleave FLN to generate a fragment that localizes with AR in the nucleus. Accumulating evidences suggest calpains function in muscle during myogenesis, including its expression during the early period of myogenic differentiation, its presence in contractile apparatus, and its involvement in the degradation of muscle fibers [55,56]. Further investigation into whether these regulations of FLN contribute to the function of AR in skeletal muscle would be worthwhile.

- ARA55, also known as hydrogen peroxide-inducible clone-5 (Hic-5), is highly similar to another AR coregulator, paxillin, that both contain amino-terminal LD motifs (contain consensus sequence LD₂LD₁) and carboxyl-terminal LIM domains [57]. The LIM domain is defined as cysteine-rich double zinc finger with the consensus sequence CX₂CX₁₆-23HX₂CX₂CX₂CX₁₆-21CX₂(C/H/D). LIM proteins are classified into four broad groups, based largely on their localization, known partners, and apparent biological function [58,59]. Group 1 LIM proteins localize primarily within the nucleus and affect transcription directly through their associated homeodomains or by acting as transcriptional cofactors. LIM proteins in this group are important regulators of development in various tissues. Group 2 LIM proteins are comprised of the LIM domain only. Four-and-a-half-LIM only proteins (FHL) belong to this group and FHL2 has been identified as an AR coactivator that expresses abundantly in cardiac muscle, but not skeletal muscle [60]. Group 3 LIM proteins contain domains associated with actin, therefore interact with the cytoskeleton to function in controlling cell adhesion, shape, motility, and trafficking of molecules. In this group, ARA55 and paxillin were identified as AR coregulators [61,62]. The fourth group of LIM proteins bears enzyme activity, such as monooxygenase and serine/threonine kinase, and can also associate with actin.

ARA55 was identified as an AR associated protein that serves as a coactivator for AR from yeast two-hybrid screening using mutant AR (threonine 877 mutated to serine) as bait [61]. The

FXXLF motifs located in the linker or next to the LIM domain are responsible for interacting with AR [63]. ARA55 can enhance the transactivity of AR, progesterone receptor, and glucocorticoid receptor (GR). Cell adhesion kinase β (CAK β)/proline-rich tyrosine kinase 2 (Pyk2) interacts and phosphorylates ARA55 at tyrosine 43 and blocks ARA55 from interaction with AR, therefore suppresses its coactivator function toward AR [64].

ARA55 was identified as a CAK β /Pyk2 binding protein that is located in the focal adhesion region, a structural link and signal transduction site between the extracellular matrix and actin cytoskeleton [57]. Nuclear-focal adhesion site shuttling of ARA55 is sensitive to redox status through the oxidant-sensitive nuclear export signal and also is dependent on its partners, focal adhesion kinase (FAK) and protein tyrosine phosphatase (PTP-PEST) [65,66]. The correlation between ARA55 expression and muscle differentiation is observed in mouse embryos and in C2C12 myoblasts where the expression level of ARA55 increased slightly at the initiation stage of differentiation and then gradually decreased until it was not detectable after differentiation. Knock-down of ARA55 by anti-sense in C2C12 cells decreased the myotube formation and reduced several markers of muscle differentiation, such as Myo D, myogenin, MHC, and p21 [67,68]. However, forced expression of ARA55 in C2C12 also blocked differentiation and induced apoptosis [68]. A recent report identified six isoforms of Hic-5 in C2C12 myoblasts that were important for myotube formation and chemodifferentiation controlled by integrins signaling [69]. The results suggested the promoting role of ARA55 during the initial stage of myogenesis and the suppressive role during progression of myogenic differentiation.

- Paxillin, a member of the group 3 subfamily of LIM domain proteins, is localized within focal adhesions and participates in a number of signal transduction pathways stimulated by growth factors, activated integrins, and angiotensin, etc. [70]. Both ARA55 and paxillin can shuttle between focal adhesion regions and the nuclear matrix. They also interact with both AR and GR through the LIM domain located in the C-terminus and function as coactivators for AR and GR, but not ER. The coactivator function is located in the C-terminal of paxillin which is different from ARA55 [62]. Paxillin is phosphorylated by focal adhesion kinase-Src (tyrosine 31 and 118), JNK (serine 178), p38 (serine 85), glycogen synthase kinase 3 (GSK-3)-ERK (serine 126/130), and kinases activated by extracellular matrix (ECM) engaged integrins (serine 188/190), cell adhesion, and angiotensin II (threonine 398/403 and serine 457/481) [71]. However, it is not clear whether these phosphorylations alter the coactivator function of paxillin.

Paxillin locates in focal adhesion regions through its LIM domains, where it acts as a scaffold protein that transmits extracellular signaling from ECM-integrins, growth factors and angiotensin II to FAK, Src, paxillin associated kinase and GSK-3 to regulate cytoskeletal rearrangements leading to the modulation of cell adhesion, spreading, and motility [70]. In skeletal muscle, it locates in costameres, myotendinous, and the neuromuscular junction, which are functional structures conducting signaling of the contraction and growth of myofibrils [72,73]. The expression of ARA55 and paxillin in muscle tissues show different patterns with paxillin generally expressed in striated muscle tissues while ARA55 expresses in smooth muscles [74]. In contrast to ARA55, overexpression of paxillin in myoblast cells increases proliferation and suppresses differentiation [75]. The expression and activation of paxillin are increased during both mechanical overloading-induced hypertrophy, and PKC downregulation-induced myogenesis [76,77]. Overall, paxillin participates in myoblast proliferation, cytoskeletal rearrange-

ments during myofibrils formation, and transducing signals mediating contraction and growth of muscles. The coactivator function of paxillin toward AR might contribute to part of these signalings in muscle.

5. Summary

Androgen is a well-known steroid promoting anabolic metabolism in skeletal muscle and widely used for enhancing muscle mass and strength. In order to magnify this effect and eliminate the proliferative effect in prostate epithelium, research has been conducted for the manufacture of SARMS that act specifically in skeletal muscle. One strategy to pursue SARM study is by identifying skeletal muscle specific/abundant coregulators of AR thereafter engineering the structures of SARMS that modulate the AR recruitment of such coregulators. By yeast two-hybrid screening of AR interacting protein from skeletal muscle cDNA library, we identified supervillin and gelsolin that are the actin-binding proteins. This finding led us to speculate the unique role of actin-binding proteins in regulating AR in skeletal muscle. Here we discussed how these AR coregulators bearing actin-binding ability participate in myogenesis and muscle function. Overall, these actin-binding proteins participate in growth, death, and signal transduction of skeletal muscle coupled with androgen-AR signaling in these development processes and functions. Therefore, targeting these coregulators might lead to the discovery of SARMS that specifically modulate AR signaling in skeletal muscle.

References

- W. Gao, P.J. Reiser, C.C. Coss, M.A. Phelps, J.D. Kearbey, D.D. Miller, J.T. Dalton, Selective androgen receptor modulator treatment improves muscle strength and body composition and prevents bone loss in orchidectomized rats, *Endocrinology* 146 (11) (2005) 4887–4897.
- J.N. Miner, W. Chang, M.S. Chapman, P.D. Finn, M.H. Hong, F.J. Lopez, K.B. Marschke, J. Rosen, W. Schrader, R. Turner, A. van Oeveren, H. Viveros, L. Zhi, A. Negro-Vilar, An orally active selective androgen receptor modulator is efficacious on bone, muscle, and sex function with reduced impact on prostate, *Endocrinology* 148 (1) (2007) 363–373.
- J. Ostrowski, J.E. Kuhns, J.A. Lupisella, M.C. Manfredi, B.C. Beehler, S.R. Krystek Jr., Y. Bi, C. Sun, R. Seethala, R. Golla, P.G. Slepsh, A. Fura, Y. An, K.F. Kish, J.S. Sack, K.A. Mookhtiar, G.J. Grover, L.G. Hamann, Pharmacological and X-ray structural characterization of a novel selective androgen receptor modulator: potent hyperanabolic stimulation of skeletal muscle with hypostimulation of prostate in rats, *Endocrinology* 148 (1) (2007) 4–12.
- Nuclear Receptors Nomenclature Committee, A unified nomenclature system for the nuclear receptor superfamily, *Cell* 97 (2) (1999) 161–3.
- H.E. MacLean, G.L. Warne, J.D. Zajac, Localization of functional domains in the androgen receptor, *J. Steroid Biochem. Mol. Biol.* 62 (4) (1997) 233–242.
- C. Chang, A. Saltzman, S. Yeh, W. Young, E. Keller, H.J. Lee, C. Wang, A. Mizokami, Androgen receptor: an overview, *Crit. Rev. Eukaryot. Gene. Expr.* 5 (2) (1995) 97–125.
- A. Moilanen, N. Rouleau, T. Ikonen, J.J. Palvimo, O.A. Janne, The presence of a transcription activation function in the hormone-binding domain of androgen receptor is revealed by studies in yeast cells, *FEBS Lett.* 412 (2) (1997) 355–358.
- B. He, J.A. Kempainen, J.J. Voegel, H. Gronemeyer, E.M. Wilson, Activation function 2 in the human androgen receptor ligand binding domain mediates interdomain communication with the NH(2)-terminal domain, *J. Biol. Chem.* 274 (52) (1999) 37219–37225.
- Z.X. Zhou, M.V. Lane, J.A. Kempainen, F.S. French, E.M. Wilson, Specificity of ligand-dependent androgen receptor stabilization: receptor domain interactions influence ligand dissociation and receptor stability, *Mol. Endocrinol.* 9 (2) (1995) 208–218.
- E.T. Keller, W.B. Ershler, C. Chang, The androgen receptor: a mediator of diverse responses, *Front Biosci.* 1 (1996) d59–d71.
- H. Poukka, U. Karvonen, O.A. Janne, J.J. Palvimo, Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1), *Proc. Natl. Acad. Sci. U.S.A.* 97 (26) (2000) 14145–14150.
- Y. Wen, M.C. Hu, K. Makino, B. Spohn, G. Bartholomeusz, D.H. Yan, M.C. Hung, HER-2/neu promotes androgen-independent survival and growth of prostate cancer cells through the akt pathway, *Cancer Res.* 60 (24) (2000) 6841–6845.
- S. Burgdorf, P. Leister, K.H. Scheidtmann, TSG101 interacts with apoptosis-antagonizing transcription factor and enhances androgen receptor-mediated transcription by promoting its monoubiquitination, *J. Biol. Chem.* 279 (17) (2004) 17524–17534.

- [14] M. Fu, C. Wang, J. Wang, X. Zhang, T. Sakamaki, Y.G. Yeung, C. Chang, T. Hopp, S.A. Fuqua, E. Jaffray, R.T. Hay, J.J. Palvimo, O.A. Janne, R.G. Pestell, Androgen receptor acetylation governs trans activation and MEKK1-induced apoptosis without affecting in vitro sumoylation and trans-repression function, *Mol. Cell Biol.* 22 (10) (2002) 3373–3388.
- [15] C.K. Glass, M.G. Rosenfeld, The coregulator exchange in transcriptional functions of nuclear receptors, *Genes Dev.* 14 (2) (2000) 121–141.
- [16] D.K. Lee, H.O. Duan, C. Chang, From androgen receptor to the general transcription factor TFIID. Identification of cdk activating kinase (CAK) as an androgen receptor NH(2)-terminal associated coactivator, *J. Biol. Chem.* 275 (13) (2000) 9308–9313.
- [17] D.M. Ozanne, M.E. Brady, S. Cook, L. Gaughan, D.E. Neal, C.N. Robson, Androgen receptor nuclear translocation is facilitated by the f-actin cross-linking protein filamin, *Mol. Endocrinol.* 14 (10) (2000) 1618–1626.
- [18] V. Boonyaratankornkit, V. Melvin, P. Prendergast, M. Altmann, L. Ronfani, M.E. Bianchi, L. Taraseviciene, S.K. Nordeen, E.A. Allegretto, D.P. Edwards, High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding in vitro and transcriptional activity in mammalian cells, *Mol. Cell Biol.* 18 (8) (1998) 4471–4487.
- [19] S. Yeh, H.K. Lin, H.Y. Kang, T.H. Thin, M.F. Lin, C. Chang, From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells, *Proc. Natl. Acad. Sci. U.S.A.* 96 (10) (1999) 5458–5463.
- [20] Y. Shang, M. Brown, Molecular determinants for the tissue specificity of SERMs, *Science* 295 (5564) (2002) 2465–2468.
- [21] H.J. Ting, Y.C. Hu, C. Chang, Actin monomer enhances supervillin-modulated androgen receptor transactivation, *Biochem. Biophys. Res. Commun.* 319 (2) (2004) 393–396.
- [22] J.Y. Dube, R. Lesage, R.R. Tremblay, Androgen and estrogen binding in rat skeletal and perineal muscles, *Can. J. Biochem.* 54 (1) (1976) 50–55.
- [23] Y. Joubert, C. Tobin, M.C. Lebart, Testosterone-induced masculinization of the rat levator ani muscle during puberty, *Dev. Biol.* 162 (1) (1994) 104–110.
- [24] A.D. Mooradian, J.E. Morley, S.G. Korenman, Biological actions of androgens, *Endocr. Rev.* 8 (1) (1987) 1–28.
- [25] M.L. Marin, M.L. Tobias, D.B. Kelley, Hormone-sensitive stages in the sexual differentiation of laryngeal muscle fiber number in *Xenopus laevis*, *Development* 110 (3) (1990) 703–711.
- [26] D.S. Catz, L.M. Fischer, M.C. Moschella, M.L. Tobias, D.B. Kelley, Sexually dimorphic expression of a laryngeal-specific, androgen-regulated myosin heavy chain gene during *Xenopus laevis* development, *Dev. Biol.* 154 (2) (1992) 366–376.
- [27] Y. Joubert, C. Tobin, Satellite cell proliferation and increase in the number of myonuclei induced by testosterone in the levator ani muscle of the adult female rat, *Dev. Biol.* 131 (2) (1989) 550–557.
- [28] Y. Joubert, C. Tobin, Testosterone treatment results in quiescent satellite cells being activated and recruited into cell cycle in rat levator ani muscle, *Dev. Biol.* 169 (1) (1995) 286–294.
- [29] R.E. Allen, K.C. Masak, P.K. McAllister, R.A. Merkel, Effect of growth hormone, testosterone and serum concentration on actin synthesis in cultured satellite cells, *J. Anim. Sci.* 56 (4) (1983) 833–837.
- [30] M.E. Doumit, D.R. Cook, R.A. Merkel, Testosterone up-regulates androgen receptors and decreases differentiation of porcine myogenic satellite cells in vitro, *Endocrinology* 137 (4) (1996) 1385–1394.
- [31] I. Sinha-Hikim, W.E. Taylor, N.F. Gonzalez-Cadavid, W. Zheng, S. Bhasin, Androgen receptor in human skeletal muscle and cultured muscle satellite cells: up-regulation by androgen treatment, *J. Clin. Endocrinol. Metab.* 89 (10) (2004) 5245–5255.
- [32] S.M. Breedlove, Cellular analyses of hormone influence on motoneuronal development and function, *J. Neurobiol.* 17 (3) (1986) 157–176.
- [33] M.L. Powers, J.R. Florini, A direct effect of testosterone on muscle cells in tissue culture, *Endocrinology* 97 (4) (1975) 1043–7.
- [34] D.K. Lee, Androgen receptor enhances myogenin expression and accelerates differentiation, *Biochem. Biophys. Res. Commun.* 294 (2) (2002) 408–413.
- [35] S. Altuwajri, D.K. Lee, K.H. Chuang, H.J. Ting, Z. Yang, Q. Xu, M.Y. Tsai, S. Yeh, L.A. Hanchett, H.C. Chang, C. Chang, Androgen receptor regulates expression of skeletal muscle-specific proteins and muscle cell types, *Endocrine* 25 (1) (2004) 27–32.
- [36] R. Singh, J.N. Artaza, W.E. Taylor, N.F. Gonzalez-Cadavid, S. Bhasin, Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway, *Endocrinology* 144 (11) (2003) 5081–5088.
- [37] V.A. Rogozkin (Ed.), *Metabolism of Anabolic Androgenic Steroids*, CRC Press Inc., United States, 1991.
- [38] K. Mulligan, M. Schambelan, Anabolic treatment with GH, IGF-I, or anabolic steroids in patients with HIV-associated wasting, *Int. J. Cardiol.* 85 (1) (2002) 151–159.
- [39] J. Zhao, W.A. Bauman, R. Huang, A.J. Caplan, C. Cardozo, Oxandrolone blocks glucocorticoid signaling in an androgen receptor-dependent manner, *Steroids* 69 (5) (2004) 357–366.
- [40] E. Kamanga-Sollo, M.S. Pampusch, G. Xi, M.E. White, M.R. Hathaway, W.R. Dayton, IGF-I mRNA levels in bovine satellite cell cultures: effects of fusion and anabolic steroid treatment, *J. Cell Physiol.* 201 (2) (2004) 181–189.
- [41] F. Hartgens, H. Kuipers, Effects of androgenic-anabolic steroids in athletes, *Sports Med.* 34 (8) (2004) 513–554.
- [42] D.J. Kwiatkowski, Functions of gelsolin: motility, signaling, apoptosis, cancer, *Curr. Opin. Cell Biol.* 11 (1) (1999) 103–108.
- [43] H. Fujita, P.G. Allen, P.A. Janmey, T. Azuma, D.J. Kwiatkowski, T.P. Stossel, N. Kuzumaki, Induction of apoptosis by gelsolin truncates, *Ann. N.Y. Acad. Sci.* 886 (1999) 217–220.
- [44] K. Nishimura, H.J. Ting, Y. Harada, T. Tokizane, N. Nonomura, H.Y. Kang, H.C. Chang, S. Yeh, H. Miyamoto, M. Shin, K. Aozasa, A. Okuyama, C. Chang, Modulation of androgen receptor transactivation by gelsolin: a newly identified androgen receptor coregulator, *Cancer Res.* 63 (16) (2003) 4888–4894.
- [45] A. Scholz, H. Hinssen, Biphasic pattern of gelsolin expression and variations in gelsolin-actin interactions during myogenesis, *Exp. Cell Res.* 219 (2) (1995) 384–391.
- [46] K.N. Pestonjamas, R.K. Pope, J.D. Wulffkuhle, E.J. Luna, Supervillin (p205): a novel membrane-associated, F-actin-binding protein in the villin/gelsolin superfamily, *J. Cell Biol.* 139 (5) (1997) 1255–1269.
- [47] S.W. Oh, R.K. Pope, K.P. Smith, J.L. Crowley, T. Nebl, J.B. Lawrence, E.J. Luna, Archvillin, a muscle-specific isoform of supervillin, is an early expressed component of the costameric membrane skeleton, *J. Cell Sci.* 116 (Pt 11) (2003) 2261–2275.
- [48] H.J. Ting, S. Yeh, K. Nishimura, C. Chang, Supervillin associates with androgen receptor and modulates its transcriptional activity, *Proc. Natl. Acad. Sci. U.S.A.* 99 (2) (2002) 661–666.
- [49] C.J. Loy, K.S. Sim, E.L. Yong, Filamin-A fragment localizes to the nucleus to regulate androgen receptor and coactivator functions, *Proc. Natl. Acad. Sci. U.S.A.* 100 (8) (2003) 4562–4567.
- [50] T.G. Thompson, Y.M. Chan, A.A. Hack, M. Brosius, M. Rajala, H.G. Lidov, E.M. McNally, S. Watkins, L.M. Kunkel, Filamin 2 (FLN2): a muscle-specific sarcolemma-interacting protein, *J. Cell Biol.* 148 (1) (2000) 115–126.
- [51] P.F. van der Ven, S. Wiesner, P. Salmikangas, D. Auerbach, M. Himmel, S. Kempa, K. Hayess, D. Pacholsky, A. Taivainen, R. Schroder, O. Carpen, D.O. Furst, Indications for a novel muscular dystrophy pathway. Gamma-filamin, the muscle-specific filamin isoform, interacts with myotilin, *J. Cell Biol.* 151 (2) (2000) 235–248.
- [52] S.C. Goetsch, C.M. Martin, L.J. Embree, D.J. Garry, Myogenic progenitor cells express filamin C in developing and regenerating skeletal muscle, *Stem Cells Dev.* 14 (2) (2005) 181–187.
- [53] A. Deshmukh, V.G. Coffey, Z. Zhong, A.V. Chibalin, J.A. Hawley, J.R. Zierath, Exercise-induced phosphorylation of the novel akt substrates AS160 and filamin A in human skeletal muscle, *Diabetes* 55 (6) (2006) 1776–1782.
- [54] U. Tigges, B. Koch, J. Wissing, B.M. Jockusch, W.H. Ziegler, The F-actin cross-linking and focal adhesion protein filamin A is a ligand and in vivo substrate for protein kinase C alpha, *J. Biol. Chem.* 278 (26) (2003) 23561–23569.
- [55] F. Raynaud, C. Jond-Necand, A. Marcilhac, D. Furst, Y. Benyamin, Calpain 1-gamma filamin interaction in muscle cells: a possible in situ regulation by PKC-alpha, *Int. J. Biochem. Cell Biol.* 38 (3) (2006) 404–413.
- [56] K.B. Kwak, S.S. Chung, O.M. Kim, M.S. Kang, D.B. Ha, C.H. Chung, Increase in the level of m-calpain correlates with the elevated cleavage of filamin during myogenic differentiation of embryonic muscle cells, *Biochim. Biophys. Acta* 1175 (3) (1993) 243–249.
- [57] M. Matsuya, H. Sasaki, H. Aoto, T. Mitaka, K. Nagura, T. Ohba, M. Ishino, S. Takahashi, R. Suzuki, T. Sasaki, Cell adhesion kinase beta forms a complex with a new member, hic-5, of proteins localized at focal adhesions, *J. Biol. Chem.* 273 (2) (1998) 1003–1014.
- [58] M. Taira, J.L. Eppard, A. Steinmetz, I.B. Dawid, Classification of LIM proteins, *Trends Genet.* 11 (11) (1995) 431–432.
- [59] J.L. Kadmas, M.C. Beckerle, The LIM domain: from the cytoskeleton to the nucleus, *Nat. Rev. Mol. Cell Biol.* 5 (11) (2004) 920–931.
- [60] J.M. Muller, U. Isele, E. Metzger, A. Rempel, M. Moser, A. Pscherer, T. Breyer, C. Holubarsch, R. Buettner, R. Schule, FHL2, a novel tissue-specific coactivator of the androgen receptor, *EMBO J.* 19 (3) (2000) 359–369.
- [61] N. Fujimoto, S. Yeh, H.Y. Kang, S. Inui, H.C. Chang, A. Mizokami, C. Chang, Cloning and characterization of androgen receptor coactivator, ARA55, in human prostate, *J. Biol. Chem.* 274 (12) (1999) 8316–8321.
- [62] M. Kasai, J. Guerrero-Santoro, R. Friedman, E.S. Leman, R.H. Getzenberg, D.B. DeFranco, The group 3 LIM domain protein paxillin potentiates androgen receptor transactivation in prostate cancer cell lines, *Cancer Res.* 63 (16) (2003) 4927–4935.
- [63] C.L. Hsu, Y.L. Chen, S. Yeh, H.J. Ting, Y.C. Hu, H. Lin, X. Wang, C. Chang, The use of phage display technique for the isolation of androgen receptor interacting peptides with (F/W)XXL(F/W) and FXXLY new signature motifs, *J. Biol. Chem.* 278 (26) (2003) 23691–23698.
- [64] X. Wang, Y. Yang, X. Guo, E.R. Sampson, C.L. Hsu, M.Y. Tsai, S. Yeh, G. Wu, Y. Guo, C. Chang, Suppression of androgen receptor transactivation by Pyk2 via interaction and phosphorylation of the ARA55 coregulator, *J. Biol. Chem.* 277 (18) (2002) 15426–15431.
- [65] M. Shibanuma, J.R. Kim-Kanayama, K. Ishino, N. Sakamoto, T. Hishiki, K. Yamaguchi, K. Mori, J. Mashimo, K. Nose, Hic-5 communicates between focal adhesions and the nucleus through oxidant-sensitive nuclear export signal, *Mol. Biol. Cell* 14 (3) (2003) 1158–1171.
- [66] M. Shibanuma, K. Mori, J.R. Kim-Kanayama, K. Nose, Involvement of FAK and PTP-PEST in the regulation of redox-sensitive nuclear-cytoplasmic shuttling of a LIM protein, hic-5, *Antioxid. Redox Signal* 7 (3–4) (2005) 335–347.
- [67] M. Shibanuma, Y. Iwabuchi, K. Nose, Possible involvement of hic-5, a focal adhesion protein, in the differentiation of C2C12 myoblasts, *Cell Struct. Funct.* 27 (1) (2002) 21–27.
- [68] Y. Hu, P.J. Cascone, L. Cheng, D. Sun, J.R. Nambu, L.M. Schwartz, Lepidopteran DALP, and its mammalian ortholog Hic-5, function as negative regulators

- of muscle differentiation, *Proc. Natl. Acad. Sci. U.S.A.* 96 (18) (1999) 10218–10223.
- [69] Z.L. Gao, R. Deblis, H. Glenn, L.M. Schwartz, Differential roles of HIC-5 isoforms in the regulation of cell death and myotube formation during myogenesis, *Exp. Cell Res.* (2007).
- [70] C.E. Turner, Paxillin interactions, *J. Cell. Sci.* 113 (Pt. 23) (2000) 4139–4140.
- [71] X. Cai, M. Li, J. Vrana, M.D. Schaller, Glycogen synthase kinase 3- and extracellular signal-regulated kinase-dependent phosphorylation of paxillin regulates cytoskeletal rearrangement, *Mol. Cell Biol.* 26 (7) (2006) 2857–2868.
- [72] C.E. Turner, N. Kramarcy, R. Sealock, K. Burrige, Localization of paxillin, a focal adhesion protein, to smooth muscle dense plaques, and the myotendinous and neuromuscular junctions of skeletal muscle, *Exp. Cell Res.* 192 (2) (1991) 651–655.
- [73] N.L. Quach, T.A. Rando, Focal adhesion kinase is essential for costamereogenesis in cultured skeletal muscle cells, *Dev. Biol.* 293 (1) (2006) 38–52.
- [74] T. Yuminamochi, Y. Yatomi, M. Osada, T. Ohmori, Y. Ishii, K. Nakazawa, S. Hosogaya, Y. Ozaki, Expression of the LIM proteins paxillin and hic-5 in human tissues, *J. Histochem. Cytochem.* 51 (4) (2003) 513–521.
- [75] S.K. Sastry, M. Lakonishok, S. Wu, T.Q. Truong, A. Huttenlocher, C.E. Turner, A.F. Horwitz, Quantitative changes in integrin and focal adhesion signaling regulate myoblast cell cycle withdrawal, *J. Cell Biol.* 144 (6) (1999) 1295–1309.
- [76] S.E. Gordon, M. Fluck, F.W. Booth, Selected contribution: skeletal muscle focal adhesion kinase, paxillin, and serum response factor are loading dependent, *J. Appl. Physiol.* 90 (3) (2001) 1174–1183, discussion 1165.
- [77] H.L. Goel, C.S. Dey, PKC-regulated myogenesis is associated with increased tyrosine phosphorylation of FAK, cas, and paxillin, formation of cas-CRK complex, and JNK activation, *Differentiation* 70 (6) (2002) 257–271.