Effects of dihydrotestosterone on skeletal muscle transcriptome in mice measured by serial analysis of gene expression

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Abstract

In order to characterize the action of androgen in skeletal muscle, we have investigated the effects of castration (GDX) and dihydrotestosterone (DHT) on global gene expression in mice. The serial analysis of gene expression method was performed in the muscle of male mice in six experimental groups: intact, GDX and GDX+DHT injection 1, 3, 6 or 24 h before they were killed. A total of 780 822 sequenced tags quantified the expression level of 80 142 tag species. Thirteen and seventy-nine transcripts were differentially expressed in GDX and DHT respectively \((P<0.05)\), including eight partially characterized and 21 potential novel transcripts. The induced transcripts within 3 h after DHT injection were involved in the following functions: transcription, protein synthesis, modification and degradation, muscle contraction and relaxation, cell signaling, polyamine biosynthesis, cell cycle progression and arrest, angiogenesis, energy metabolism and immunity. However, the inductions of transcripts related to cell cycle arrest and angiogenesis were no longer significant 24 h after DHT injection. The current study might suggest that DHT promotes protein synthesis, cell signaling, cell proliferation and ATP production, as well as muscle contraction and relaxation at the transcriptional level in skeletal muscle \textit{in vivo}.

Introduction

Sarcopenia, characterized by a loss of muscle mass, strength and endurance, occurs with aging and chronic medical disorders such as human immunodeficiency virus (HIV) infection and long-term systemic glucocorticoid (Gcc) therapy. On the other hand, testosterone supplementations in older men and HIV-infected men with weight loss who have low testosterone concentrations (Bhasin et al. 2001) as well as men requiring long-term systemic Gcc treatment (Truhan & Ahmed 1989) increase fat-free mass and muscle strength.

Skeletal muscle is one of the target tissues for the anabolic action of androgens. Androgen receptors (AR), localized in the muscle cells as well as fat, nerve and mesenchymal pluripotent cells which reside in the muscle tissue, likely mediate the effects of androgens by increasing muscle mass, protein synthesis, ribosomal contents, mitochondrial areas, myonuclear number, satellite cell number and myogenesis of pluripotent mesenchymal cells while decreasing protein degradation and adipogenesis of the pluripotent mesenchymal cells (Herbst & Bhasin 2004). Upon the ligand binding to intracellular AR, the androgen–AR complex is translocated to the nucleus and binds to specific DNA sequences, androgen response elements, which results in transcription of specific genes (Michel & Baulieu 1980, Simental et al. 1991). Androgens also have rapid non-genomic actions in the muscle (Estrada et al. 2000, 2003), including membrane receptor coupled to G protein, inositol 1,4,5-trisphosphate ([IP]$_3$) receptor, calcium ion (Ca$^{2+}$) and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) phosphorylation cascade. In addition to its role in muscle contraction, intracellular Ca$^{2+}$ is thought to regulate gene expression in the skeletal muscle (Estrada et al. 2001, Araya et al. 2003). Thus, both genomic and non-genomic actions of androgens are responsible for the transcription of androgen responsive genes (ARG).

Circulating testosterone is transformed into its bioactive metabolite, dihydrotestosterone (DHT) by 5$\alpha$-reductase in target tissues. DHT is one of the most potent natural androgens. However, the molecular mechanisms of the anabolic effect of androgens in skeletal muscle are poorly understood. With the advent of serial analysis of gene expression (SAGE) (Velculescu et al. 1995), new possibilities have arisen for large scale transcriptome analysis. Using this method, we have previously studied the molecular mechanisms responsible for muscle atrophy caused by immobilization in rats (St-Amand et al. 2001), as well as the gene expression profile of endurance-trained men (Yoshioka et al. 2003).
In the present study, we have investigated the effects of castration (GDX) and DHT on global gene expression in the skeletal muscle of male mice using the SAGE strategy. DHT-modulated transcripts are involved in Ca²⁺ release, cell signaling, cell proliferation, mRNA and protein synthesis and energy metabolism. These findings constitute a first step towards a precise understanding of the molecular mechanisms involved in the physiological effects of androgens in skeletal muscle.

Materials and methods

Sample preparation

The right gastrocnemius muscle was obtained from C57BL6 mice of 12 to 14 weeks of age purchased from Charles River Canada Inc. (St-Constant, QC, Canada). The animals were housed with lights on from 0715 to 1915 h, and had access to Lab Rodent Diet No. 5002 (Ren's Feed and Suppliers, Oakville, ON, Canada) and water ad libitum. All treatment was performed on 26 intact mice. GDX was performed 7 days prior to organ collection in each of the 14 mice from the GDX and DHT groups. Mice of the GDX group received an i.p. injection of vehicle solution (0·4% (w/v) Methocel A15 LV Premium/5% ethanol; Dow Chemicals Co, Laval, Quebec, Canada) 24 h before they were killed, while a physiological dose of DHT (0·1 mg/mouse) was injected 1, 3, 6 and 24 h prior to their being killed (DHT 1 h, DHT 3 h, DHT 6 h and DHT 24 h groups). All mice were killed between 0830 and 1230 h by cervical dislocation and decapitation under isoflurane anesthesia. The right gastrocnemius muscle was sampled from each mouse and pooled together for analysis of the same group to eliminate inter-individual variations and to extract sufficient amounts of mRNA. The tissues were stored at −80°C until RNA extraction. All animal experimentation was conducted in accordance with the requirements of the Canadian Council on Animal Care.

Transcriptome analysis

The SAGE method was performed as previously described (Veclucescu et al. 1995, St-Amand et al. 2001). Total RNA was isolated by Trizol (Invitrogen Canada Inc., Burlington, ON, Canada). The quality of total RNA was monitored by micro-capillary electrophoresis (Bioanalyzer 2100; Agilent Technologies, Mississauga, ON, Canada). Polyadenylated RNA was extracted with the Oligotex mRNA Mini Kit (Qiagen Inc., Mississauga, ON, Canada). annealed with the biotin-5'-T18-3' primer and converted to cDNA using the cDNA synthesis kit (Invitrogen Canada Inc.). The resulting cDNAs were digested with NlaIII (New England BioLabs Ltd, Pickering, ON, Canada) and the 3' restriction fragments were isolated with streptavidin-coated magnetic beads (Dynal Biotech LLC, Brown Deer, WI, USA) and separated into two populations. Each population was ligated to one of two annealed linkers and extensively washed to remove unligated linkers. The tag beside the most 3' NlaIII restriction site (CATG) of each transcript was released by digestion with BsmFI (New England BioLabs Ltd). The blunting kit from Takara Bio Inc. (Otsu, Japan) was used for the blunting and ligation of the two tag populations. The resulting ligation products containing the ditags were amplified by PCR and digested with NlaIII. The band containing the ditags was extracted from the 12% polyacrylamide gel with Spin-X microcentrifuge tube (Fisher, Pittsburgh, PA, USA). The purified ditags were self-ligated to form concatemers using T4 ligase (Invitrogen Canada Inc.). The concatemers ranging from 500 bp to 1800 bp were isolated by agarose gel and extracted with Gene-Clean Spin (QiBiogene, Montreal, QC, Canada). The resulting DNA fragments were ligated into the SphI site of pUC19 and cloned into UltraMAX DH5αFT competent cell (Invitrogen Canada Inc.). White colonies were picked up with a Q-Bot colony picker (Genetix Ltd, New Milton, Hants, UK). Concatemer inserts were sequenced by the Applied Biosystems 3730 (Foster City, CA, USA).

Bioinformatic analysis

Sequence files were analyzed using the SAGEana program, a modification of SAGEparser (Dinel et al. 2005). In brief, tags corresponding to linker sequences were discarded and replicate concatemers were counted only once. Identification of the transcripts was obtained by matching the 15 bp (sequence at the last CATG +11 bp tags) with SAGEmap, UniGene and GenBank databases on 5 March 2004. A minimum of one expressed sequence tag (EST) with a known polyA tail had to be in the UniGene cluster to identify the last NlaIII site on the corresponding cDNA. We have previously shown that the SAGE method is very reproducible with $r^2 = 0.96$ between two libraries constructed from the same total RNA pool (Dinel et al. 2005). Classification of the transcripts was based upon the updated information of the genome directory (Adams et al. 1995) found at the TIGR web site (http://www.tigr.org/) as well as previously published papers.

Statistical analysis

We used the comparative count display test to identify the transcripts significantly differentially expressed ($P \leq 0·05$) between the groups with more than a twofold change (Lash et al. 2000). The data were normalized to 100 000 tags for presentation.
Results

The sequencing of 780,822 SAGE tags quantified the expression level of 80,142 tag species. In total, 13 and 79 transcripts were differentially expressed between intact vs GDX and DHT vs GDX respectively (P<0.05). Five transcripts were modulated by both GDX and DHT, in which the effects of GDX on four of these transcripts were counteracted by DHT. GDX removes all testicular hormones, precursors and secreted factors as well as their interactions, whereas we administered only the most potent natural androgen, DHT, in GDX mice. This might therefore explain the discrepancy of the effects of GDX or DHT in the transcripts regulated by only one of these experimental conditions. Moreover, the SAGE method may not have detected the down-regulation of some low expressed transcripts because of a weak statistical power (Dinel et al. 2005).

Indeed, 72% of the DHT up-regulated transcripts were not statistically detected as being regulated by GDX, since the intact group had less than nine tags. On the other hand, the changes in the expression level of some transcripts may be counteracted by a change in other isoforms and transcript species such as we have observed for S-adenosylmethionine decarboxylase 1 (Amd1). This could point to a different mechanism with respect to both up-regulation and suppression.

The transcripts related to muscle contraction, extracellular matrix (ECM), transcription and protein metabolism which were regulated by GDX and DHT are presented in Table 1. The transcripts related to both muscle contraction (triadin (Trdn)) and relaxation (parvalbumin (Pvalb)) were up-regulated by DHT injection. The expressions of serine (or cysteine) proteinase inhibitor clade A members 1a, 1b, 1e and 3k (Serpina1a, 1b, 1e and 3k) which inhibit the degradation of ECM components, were decreased by GDX whereas DHT increased EST procollagen type I (Cola2). DHT induced the expression of transcription modulators (fragile X mental retardation gene 2 (Fxr2h), O-linked N-acetylglucosamine transferase (Ogt) and SET and MYND domain containing 2 (Smyd2)). DHT up-regulated the transcripts related to ribosomal proteins (L51 (Mrpl51), L34 (Rpl34) and S20 (Rps20)), chaperones (chaperone ABC1 activity of bc1 complex-like (Cabc1) and chaperonin subunit 8 (Cct8)) and protein degradation (proteasome 26S subunit ATPase 3 (Psme3) and protease 26S subunit ATPase 5 (Psme5)), while ribosomal proteins S24 (Rps24) and S27 (Rps27) were down-regulated.

Table 2 shows the differentially expressed transcripts which are involved in cell proliferation and signaling as well as other functions. GDX down-regulated growth arrest and DNA damage-inducible 45γ (Gadd45g) and the effect was reversed by DHT treatment. DHT also increased the transcripts related to the cell proliferation, such as aldo-keto reductase family 1 member A4 (Akrla4), Golgi reassembly stacking protein 2 (Gorasp2), pituitary tumor-transforming 1 (Pttg1) and t-complex testis-expressed 1 (Tctex1), while decreasing lectin galactose-binding soluble 1 (Lgals1) which inhibits cell proliferation. Moreover, GDX and DHT modulated key transcripts related to polyamine biosynthesis (ornithine decarboxylase antizyme (Oaz), Amd1 and spermine-binding protein (Sbp)). The transcripts related to the cell signaling, such as ankyrin repeat and SOCS box-containing protein 5 (Asb5), calcium/calmodulin-dependent protein kinase IIγ (Camk2g), dual specificity phosphatase 1 (Dusp1), histidine triad nucleotide binding protein 1 (Hint1) and membrane protein palmitoylated 6 (Mpp6) were also induced by DHT. In addition, GDX down-regulated transfhyretin (Ttr) whereas DHT up-regulated β2-microglobulin (B2m), CD59a antigen (Cd59a) and dendritic cell protein GA17 (Ga17).

The modulated transcripts related to energy metabolism are presented in Table 3. In the lipid metabolism, the expression of apolipoprotein A-II (Apoa2) was decreased by GDX whereas lipin 1 (Lpin1) was increased by DHT. GDX also suppressed cytochrome c oxidase 1 (MtCo1) expression whereas DHT induced the expression of 18 transcripts related to mitochondrial oxidative phosphorylation (OxPhos) and ATP production with two exceptions (NADH dehydrogenase 3 (MtNd3) and NADH dehydrogenase 1β subcomplex 9 (Ndufb9)) which were suppressed by DHT. GDX and DHT also modulated the expression of seven partially characterized and 21 potentially novel transcripts (Table 4).

Discussion

Protein metabolism

Anabolic hormones stimulate muscle growth mainly by increasing protein synthesis (Rooyackers & Nair 1997). In this study, GDX repressed the expression of heat shock protein family member 7 (Hspb7), whereas DHT injection up-regulated five genes encoding ribosomal proteins and chaperones (Mrpl51, Rpl34, Rps20, Cct8 and Cabc1) within 3 h as well as modulating three other transcripts (Fxr2h, Rps24 and Rps27) at 24 h. In addition, to protein synthesis, Rpl34 and Rps20 are implicated in polyamine biosynthesis (Panagiotidis et al. 1995). The Cct8 protein whose expression is highly dependent on cell growth (Yokota et al. 1999) folds newly synthesized proteins including cellular components necessary for cell growth (Thulasiraman et al. 1999) as well as behaving as microtubule-associated protein (Roobol et al. 1999). Mrpl51 is encoded by mitochondrial DNA, and Cabc1 encodes a mitochondrial protein essential for the proper conformation and functioning of protein complexes in the respiratory chain (Iizumi et al. 2006).
Table 1 Components of muscle contraction, extracellular matrix, transcription and protein metabolism regulated by GDX and DHT injection in skeletal muscle

<table>
<thead>
<tr>
<th>Tags . . .</th>
<th>Intact GDX</th>
<th>GDX+DHT</th>
<th>GDX+DHT</th>
<th>GDX+DHT</th>
<th>GDX+DHT</th>
<th>GDX+DHT</th>
<th>Description</th>
<th>(UniGene cluster, GenBank accession no.)</th>
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<td>124</td>
<td>465</td>
<td>1066</td>
<td>154</td>
<td>506</td>
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<td>17</td>
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<td>5</td>
<td>3</td>
<td>15</td>
<td>EST procollagen type I α2 (Mm.277792, CB575147)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Serine (or cysteine) proteinase inhibitor clade A member 1A (Mm.259233, NM_009243), 1B (Mm.275860, NM_009244) and 1E (Mm.89843, AK004999)</td>
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<td>0</td>
<td>Serine (or cysteine) proteinase inhibitor clade A member 3K (Mm.291706, BC016407)</td>
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<td>1</td>
<td>5</td>
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<td>0</td>
<td>16</td>
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<td>1</td>
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<td>3</td>
<td>7</td>
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<td>Ribosomal protein</td>
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<td>5</td>
<td>32</td>
<td>6</td>
<td>12</td>
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<td>242</td>
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<td>159</td>
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<td>256</td>
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<td>396</td>
<td>419</td>
<td>457</td>
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<td>16</td>
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<td>17</td>
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<td>Heat shock protein family member 7 (Mm.46181, NM_013868)</td>
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<td>Degradation</td>
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<td>12</td>
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<td>Protease 26S subunit ATPase 5 (Mm.272919, NM_008950)</td>
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<td>Protease 26S subunit ATPase 3 (Mm.289832, NM_008948)</td>
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† † Significantly up- and down-regulated compared with GDX; P<0.05. Mm, mus musculus.
Indeed, the expressions of 18 transcripts related to OxPhos and ATP production were up-regulated by DHT in the current study. These data suggest that DHT increases protein synthesis and stabilization in parallel with cell growth within 3 h in mice in vivo.

Degradation of proteins by the 26S proteasome is essential for cell cycle progression, polyamine metabolism and class I heavy chain of major histocompatibility complex (MHC) presentation on the cell surface. DHT induced Psmc3 and Psmc5 whose proteins are the components of proliferation, cell signaling and other functions regulated by GDX and DHT injection in skeletal muscle, as shown in Table 2.

Table 2: Components of proliferation, cell signalling and other functions regulated by GDX and DHT injection in skeletal muscle

<table>
<thead>
<tr>
<th>Tags . . .</th>
<th>Intact GDX</th>
<th>GDX+DHT</th>
<th>Description (UniGene cluster, GenBank accession no.)</th>
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<tr>
<td>ATTACGGTGGA</td>
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<td>Gorasp2 (Mm.271950, NM_027352)</td>
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<td>32↑ 15 17↑ 5</td>
<td>Gadd45g (Mm.43831, NM_008496)</td>
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<td>264 257 243 117↑</td>
<td>Lgals1 (Mm.41288, BC046241)</td>
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<td>Pttg1 (Mm.1948, NM_011321; Mm.46248, AK009406)</td>
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<td>ACTTCAGCCAG</td>
<td>1 3</td>
<td>15 31↑ 9 21↑</td>
<td>Tctex1 (Mm.235182, BC019162)</td>
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<td>TCGTGATTTGT</td>
<td>8 14</td>
<td>35 134↑ 8 36</td>
<td>Ornithine decarboxylase antizyme (Mm.68465, NM_008753; Mm.298880, BY68448; Mm.289880, BU520342)</td>
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<td>5 12 22 26</td>
<td>Amd1 (Mm.296762, NM_007444)</td>
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<td>35 64 70↑ 141↑</td>
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↑↓ Significantly up- and down-regulated compared with GDX; P<0.05.
integral components of the 19S regulatory subunit of the 26S proteasome, which might reflect the induction of cell proliferation and modulation of immunity by DHT as discussed below.

Transcription
Transcriptional regulation is an essential control point for diverse cellular functions such as cell proliferation, differentiation, transformation and apoptosis. DHT up-regulated five transcriptional factors (Ogt, Pttg1, Psmc3, Psmc5 and Smyd2) within 3 h after the treatment as well as Fxr2h at 24 h. The activation of the MAPK cascade results in the translocation of cytoplasmic Pttg1 protein into the nucleus (Pei 2000) where Pttg1 protein transactivates target genes which promote cell proliferation (Pei 2001). The proteins of Psmc3 and Psmc5 suggest roles in the transactivation of thyroid...
hormone receptor (Ishizuka et al. 2001). GDX also decreased the expression level of Ttr, whose product is a homotrimeric plasma protein which carries thyroxine as well as retinol. Although thyroid hormones are essential during growth, both an excess and a deficiency cause muscle wasting by unknown mechanisms (Rooyackers & Nair 1997). Fxr2h protein shows a strong transcription activation (Hillman & Gecz 2001). Nuclear and cytoplasmic protein glycosylation is a widespread and reversible post-translational modification in eukaryotic cells. Intracellular glycosylation of serine and threonine residues is catalyzed by the protein of Ogt, which regulates a number of cellular functions including transcriptional activation (p53 target genes)/repression (RNA polymerase II) and translational activation (Wells et al. 2003). The presence in the Smyd2 gene of SET and MYND domains would be in agreement with effects on histone deacetylation and methylation respectively (Sims et al. 2002). Thus, our results suggest that at least some of the actions of DHT occur through activation or repression of the transcriptional regulators.

Calcium ion, muscle contraction and cell proliferation

In the skeletal muscle, Ca^{2+} plays a key role in contraction and relaxation. The present study showed that DHT treatment increased the expression of Pvalb, a high affinity Ca^{2+}-binding protein acting as a muscle relaxing factor after contraction, and Trdn which forms a quaternary complex with ryanodine receptor, junctin and calsequestrin in the lumen of sarcoplasmic reticulum (SR) for the passive buffering of SR luminal Ca^{2+} as well
as an active Ca\textsuperscript{2+} release from SR process during excitation–contraction coupling. Transgenic mice over-expressing Trdn1 in the heart show cardiac hypertrophy with impaired relaxation and blunted contractility (Kirchhefer et al. 2001). Thus, induction of both muscle relaxing and contracting factors might contribute to a power generation which is generally observed in athletes who are taking anabolic steroids.

Depolarization of muscle cells also results in a slow transient release of Ca\textsuperscript{2+}, which is mediated by phospholipase C (PLC) and IP\textsubscript{3} via IP\textsubscript{3} receptors (Estrada et al. 2001, Powell et al. 2001), and leads to the phosphorylation of ERK1/2 (Powell et al. 2001). In osteoblasts, DHT activates G\textsubscript{B\textsubscript{4}} protein coupled to PLC-\(\beta\), which increases formation of IP\textsubscript{3} and diacylglycerol (DAG), and triggers the releases of intracellular Ca\textsuperscript{2+} from endoplasmic reticulum (Zagar et al. 2004). The increases in DAG and Ca\textsuperscript{2+} levels regulate the activity of protein kinase C (PKC) which stimulates ERK1/2 via the activation of MAPK kinase 1/2 (Zagar et al. 2004). The current results have shown the inductions of Camk2g, Dusp1 and Hint1 within 3 h after DHT injection. Multifunctional Ca\textsuperscript{2+}/calmodulin-independent protein kinase II (CaMKII) mediates cellular responses to intracellular Ca\textsuperscript{2+} and is implicated in the control of essential functions such as synaptic transmission, ion channels, gene transcription and cell cycle progression (Santella 1998, Anderson 2005). Proliferating cells (Tombes & Krystal 1997) and hypertrophied heart with enhanced contractility (Colomer et al. 2003) express CaMKII\(\gamma\) isofrom which is encoded by Camk2g. Dusp1 (also named CL100 or MAPK phosphatase 1) was originally identified as an immediate early gene induced by mitogens (Charles et al. 1992, Keyse & Emslie 1992), and its transcription level reflects the activation of ERK1/2 (Camp et al. 2000). Hint1, a PKC-interacting protein which was originally thought to inhibit PKC, may play a tumor-suppressor role (Su et al. 2003). We have observed the up-regulation of Mpp6, Oaz, Psmc3 and Psmc5 within 3 h by DHT treatment. The function of Mpp6, a member of the p55-like membrane-associated guanylate kinase (MAGUK) sub-family, is not yet known. However, MAGUK interacts with glutamate receptors and various ionic channels (Godreau et al. 2004). Polyamines (spermine, spermidine and putrescine) also interact with certain ion channels and control intracellular Ca\textsuperscript{2+} levels (Williams 1997). The polyamine biosynthesis in mammalian cells begins with a production of putrescine by ornithine decarboxylase (ODC). When intracellular polyamine levels increase, ODC antizyme, encoded by Oaz, binds to ODC and facilitates its rapid degradation by the 26S proteasome (Thomas & Thomas 2003). Thus, the inductions of Oaz as well as Psmc3 and Psmc5, which are integral components of the 26S proteasome, may reflect the increased level of intracellular polyamines.

Moreover, the modulations of these transcripts as well as Mpp6 within 3 h, the same time-course as Camk2g, suggest their participation in controlling intracellular Ca\textsuperscript{2+}. In addition, the Ras/MAPK pathway controls the transcription of Cct8 (Yamazaki et al. 2003) which was induced at 1 h after DHT treatment in the present study. Taken together, the second messenger, namely intracellular Ca\textsuperscript{2+}, and its downstream cascades including PKC and MAPK, which are essential for the regulation of cell growth, seem to be modulated by DHT.

Satellite cells/myoblasts within the skeletal muscle tissue proliferate upon exposure to growth factors and following muscle injury, but they stop dividing once they fuse to pre-existing muscle fibers. The fusion is generally coupled with onset of cell proliferation. In the present study, DHT up-regulated transcripts related to S-phase entry (Pttg1) (Nasmyth et al. 2000), microtubule assembly (Cct8) (Roobol et al. 1999), bipolar spindle formation (Tctex1) (Vaisberg et al. 1993), equal chromosomal segregation (Pttg1) (Nasmyth et al. 2000), stacking of Golgi cisternae (Gorasp2) (Shorter et al. 1999) and detoxification of reactive metabolites produced during cell proliferation (Akr1a4) (Barski et al. 2004), suggesting an induction of cell proliferation by DHT. On the other hand, DHT down-regulated a cystostatic factor, Lgals1, which maintains G\textsubscript{0} and controls G\textsubscript{2} traverse (Wells & Mallucci 1991). The mitotic spindle requires the assembly/disassembly of microtubules and action of motor complex such as dynein (Vaisberg et al. 1993). Tctex1 protein is a light chain of the dynein motor complex (Tai et al. 1998). The Cct8 increases during G\textsubscript{2}/S transition through the early S phase (Yokota et al. 1999). Pttg1, human securing, accumulates at the onset of the S phase and peaks at the G\textsubscript{2}–M phases, and prevents premature separin activation during mitosis (Nasmyth et al. 2000).

DHT up-regulated Psmc3, Dusp1, Gadd45g and Pttg1 in the present conditions. Overexpression of Psmc3 increases p53 and p21 proteins (Pollice et al. 2004). In response to DNA damage and other stresses, the tumor suppressor p53 induces either cell cycle arrest or apoptosis depending on specific cellular contexts (Yu & Zhang 2005). In response to DNA damage, p33 promotes DNA repair by affecting the DNA excision repair pathway and by arresting cells in G\textsubscript{1} through induction of p21 which contribute to providing more time for the repair (Smith & Seo 2002), whereas p33-mediated G\textsubscript{1} arrest also occurs through induction of Dusp1 in the absence of DNA damage (Li et al. 2003). Protein encoded by Gadd45g interacts with p21 and suppresses cell growth without any evidence of apoptosis (Nakayama et al. 1999). The growth arrest mediated by the cell cycle inhibitors p21 and Gadd45 inhibit the apoptotic response induced by apoptotic targets of p33 (Yu & Zhang 2005). Moreover, the securing encoded by Pttg1 inhibits the ability of p33 to induce cell death.
(Bernal et al. 2002). Taken together, DHT might promote G1 arrest without inducing apoptosis in the current study.

The current study reports the induction of Amd1 which encodes S-adenosylmethionine decarboxylase (SAMDC), Oaz, Rps20 and Rpl34 as well as Psmc3 and Psmc5 after DHT injection. On the other hand, DHT down-regulated Sbp whose product correlates with spermine accumulation (Moruzzi et al. 1982). Polycationic compounds synthesized by the rate-limiting enzymes, ODC and SAMDC, are crucial for growth and proliferation of mammalian cells. The ODC antizyme encoded by Oaz, as well as ribosomal proteins L34 and S20, inhibit ODC and arginine decarboxylases (Pangiotidis et al. 1995). The ODC and SAMDC are degraded by the 26S proteasome (Yerlikaya & Stanley 2000) which is encoded by Psmc3 and Psmc5. The ODC antizyme also inhibits polyamine uptake and stimulates excretion (Sakata et al. 2000). Moreover, polyamine uptake is inhibited by PKC and is stimulated by its inhibition (Dot et al. 2000). Coincidentally, Hint1, which inhibits PKC, had a similar pattern of modulation to that of Oaz with DHT treatment. Taken together, the modulations of Pttg1, Cct8, Tctex1, Gorasp2, Akr1a4, Lgals1, Amd1, Oaz, Rpl34, Rps20, Psmc3, Psmc5 and Sbp by DHT treatment in the present study might reflect proliferation of satellite cells/myoblasts in the skeletal muscle.

In addition, Pttg1 protein induces angiogenesis both in vitro and in vivo (Ishikawa et al. 2001). In the present study, DHT up-regulated Pttg1 and Asb5 which is a novel protein implicated in the initiation of arteriogenesis (Boengler et al. 2003). Vascularization is an important determinant of energy supply and waste removal during muscle contraction, and its stimulation by DHT in this study is thus in agreement with other data presented above.

**Metabolism**

In lipid metabolism, GDX shut down Apoa2 and DHT up-regulated Lpin1. Apolipoprotein A-II encoded by Apoa2, the second most abundant protein of high density lipoprotein (HDL) particles, exerts a marked effect on HDL binding and selective lipid uptake by class B scavenger receptors. In the mouse, enhanced Lpin1 expression in the skeletal muscle promotes obesity by decreasing whole body energy expenditure and fat utilization as well as by inducing insulin resistance (Phan & Reue 2005). In contrast to what happens in the muscle, overexpression of Lpin1 in the adipose tissue causes obesity without insulin resistance (Phan & Reue 2005). We have previously reported that the expression level of Lpin1 in adipose tissue remains unaltered with DHT treatment (Bolduc et al. 2004). The induction of Lpin1 only in the muscle might suggest that carbohydrate was used for the increased OxPhos and ATP production. Further studies will be needed to clarify this intriguing mechanism.

**Immunity**

DHT increased B2m, Cd59a and Ga17 in the present study. The human homologue of Ga17 (also named hfl-B5) encodes a cell surface membrane fusion protein which enables entry and infection of herpes simplex virus (Perez et al. 2005). β2M encoded by the B2m gene is a small immunoglobulin-like protein which is non-covalently associated with the class I heavy chain of MHC molecules. Human skeletal muscle fibers do not show cytochemically demonstrable expression of class I MHC or β2M while these proteins are regularly present in regenerating muscle after chronic partial denervation, Duchenne dystrophy and several types of inflammatory myopathy (Karpati et al. 1988). Moreover, β2M is a bone-derived growth factor which stimulates bone DNA and collagen synthesis (Canalis et al. 1987). Although the effects of β2M in the skeletal muscle are not known, the present study has reported the up-regulation of B2m and EST Colla2 24 h after DHT injection. Coincidentally, Psmc3 which suppresses HIV Tat-mediated transactivation (Nelbock et al. 1990) was also up-regulated, whereas HIV Tat is known to repress the transcription of the β2M promoter (Carroll et al. 1998). Thus, the up-regulation of Psmc3 by DHT might have induced B2m and EST Colla2 in the current conditions. The myogenic process is heavily dependent upon cell migrations and adhesions. Collagen, an adhesive ECM component, binds to substrates like fibronectin, promoting cell–substrate adhesion which is a requisite for cell migration. During proliferation, satellite cells adhere to their substrate by expressing high levels of collagen type I and are beginning the process of cell migration, which leads to myoblast alignment and subsequent fusion (Velleman & McFarland 1999). Thus, the modulations of B2m and EST Colla2 by DHT treatment might suggest that myoblast proliferation (migration and adhesion) occurs 24 h after DHT stimulation in mice in vivo. In order to prevent a complement-mediated attack, host tissue expresses a variety of regulatory proteins that limit damage to the pathogen cell surface. The complement regulatory protein CD59 is a glycosyl-phosphatidylinositol–anchored membrane protein which inhibits the formation of membrane attack complex (MAC) and protects the cells from MAC-induced lysis (Turnberg & Botto 2003). In comparison with control muscle, atrophied muscle fibers of inflammatory myopathic patients do not contain CD59 whereas regenerating muscle cells (myoblasts and myotubes) from the same patients have a strong immunostaining with CD59 antibody (Gendek-Kubiak & Gendek 2004). From the facts that (1) β2M and CD59 are commonly highly expressed in myoblasts and regenerating fibers,
(2) muscle regeneration induced by muscle injury and hypertrophy induced by growth stimuli share common processes of muscle growth and (3) many transcripts modulated at 24 h after DHT treatment in the current study were related to cell proliferation (mitosis), the elevated expression levels of B2m and Cd59a 24 h after DHT injection might reflect myoblast proliferation.

Figure 1 Overview of ARGs in the skeletal muscle. The up- and down-regulated transcripts by DHT treatment are shown in red and blue (underlined) characters respectively. Transcripts showing only early (DHT 1, 3 and 6 h) response are noted as (E) or (L) respectively. The solid and dotted lines represent activation/induction and inhibition, respectively. Akr1a4, aldo-keto reductase family 1 member A4; Amd1, S-adenosylmethionine decarboxylase 1; Asb5, ankyrin repeat and SOCs box-containing protein 5; Atp5j2, ATP synthase mitochondrial F0 complex subunit f isoform 2; Atp6, ATP synthase 6; Atpaf1, ATP synthase mitochondrial F1 complex assembly factor 1; B2m, β2 microglobulin; Cabc1, chaperone ABC1 activity of bc1 complex like; Camk2g, calcium/calmodulin-dependent protein kinase IIγ; Cct8, chaperonin subunit 8; Cds9a, CDS9a antigen; Cola2, procollagen type I α2; Cyc1, cytochrome c-1; Cyp27a1, cytochrome P450 family 27 subfamily a polypeptide 1; DAG, diacylglycerol; Dusp1, dual specificity phosphatase 1; EST, expressed sequence tags; Fxr2h, fragile X mental retardation gene 2; Ga17, dendritic cell protein GA17; Gadd45g, growth arrest and DNA damage-inducible 45γ; Gorgasp2, Golgi reassembly stacking protein 2; Hint1, histidine triad nucleotide-binding protein 1; Ip3, inositol 1,4,5-trisphosphate; Lgals1, lectin galactose-binding soluble 1; Lpin1, lipin 1; MAPK, mitogen-activated protein kinase; Mrpl51, ribosomal protein L51; Mtco1, cytochrome c oxidase 1; Mtco2, cytochrome c oxidase 2; Mtco3, cytochrome c oxidase 3; Mtnd2, NADH dehydrogenase 2; Mtnd3, NADH dehydrogenase 3; Mtnd4, NADH dehydrogenase 4; Ndufa5, NADH dehydrogenase 1α subcomplex 5; Ndufb2, NADH dehydrogenase 1β subcomplex 2; Ndufb9, NADH dehydrogenase 1β subcomplex 9; Oaz, ornithine decarboxylase antizyme; Ogt, O-linked N-acetylglucosamine transferase; Pkc, protein kinase C; Plc, phospholipase C; Psmt, proteasome 26S subunit ATPase; Pvalb, parvalbumin; Rp, ribosomal protein; Pttg1, pituitary tumor-transforming 1; Sbp, spermine-binding protein; Tctex1, t-complex testis expressed 1; Tp01, polyamine transporter; Trdn, triadin.

Conclusion

An overview of ARG in skeletal muscle is presented in Fig. 1. The early responses to DHT injection (DHT 1, 3 and 6 h) are the induction of both muscle relaxing (Pvalb) and contracting (Trdn) factors which modulate intracellular Ca^{2+} levels. DHT also induced the
transcripts related to cell signaling such as Ca\(^{2+}\) (Camk2g), PKC (Hint1) and MAPK pathways (Dusp1) as well as polyamine biosynthesis (Amid1, Oaz, Psmc3, Rps20 and Rpl34), cell proliferation (Akr1a4, Cct8, Ptg1 and Tctex1), cell cycle arrest (Gadd45g), p53 (Cabc1, Dusp1 and Ptg1) and angiogenesis (Amd1, Oaz, Psmc3). The induction of mRNAs related to transcription (Ogt, Psmc3 and Psmc5), protein synthesis (Mrpl51, Ogt, Rps20 and Rpl34), modification (Cabc1, Cct8 and Ogt) and degradation (Psmc3 and Psmc5), oxidative phosphorylation (Cyc1, Mtco1, Mtco2, Cyp27a1, Ndufa5 and Ndufa2), ATP production (Atp5j2 and EST Atpa1), and lipid metabolism (Lpin1) as well as immunity (Cd59a and Ga17) have also been observed at these time-points. However, the induction of transcripts related to Ca\(^{2+}\) signaling, MAPK, cell cycle arrest, p53, protein synthesis and angiogenesis are not significant any more 24 h after DHT injection while transcripts related to cell cycle progression are still up-regulated. Moreover, the transcripts related to protein synthesis (Rps24 and Rps27) were down-regulated. These results indicate that DHT injection induces power generation, protein synthesis, mitochondrial function and satellite cell/myoblast proliferation at the transcriptional level in vivo, supporting previous findings of an anabolic action of androgen.

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