TRANSLATING SCIENCE TO BETTER HEALTH: 
THE POWER OF DIVERSITY AND 
MULTICULTURAL ENGAGEMENT

CONCURRENT SCIENTIFIC SESSION 2 
Efficacy, Safety, Side Effects, and Benefits of Complementary & Alternative Medicine

JOSE GINEL RODRIGUEZ 
Universidad Central del Caribe
VERNONIA AMYGDALINA AFFECTS INITIATION AND REGRESSION OF XENOGRAPHS

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• Validation of Vernonia amygdalina (VA)
• VA compared to two other chemotherapeutic drugs

Concurrent Scientific Session 2
Efficacy, Safety, Side Effects, and Benefits of Complementary & Alternative Medicine

Introduction
• This partnership represents a scientific collaborative effort between Jackson State University (JSU) and Charles Drew University (CDU).
• Dr. Howard and a team at JSU have expertise in working with Vernonia amygdalina (VA), an African medicinal plant known for producing anticancer effects.
• Dr. Shehla Pervin at CDU in Los Angeles, CA conducted experiments with mammary cancer stem cells (MCSCs) in nude mice.
• Working in partnership, we generated preliminary preclinical data to support the development of phase I or II clinical hypotheses.
Introduction (cont’d)

Flower and leaves of V. amygdalina
Entire VA plant

Background

- Worldwide, breast cancer continues to claim the lives of billions of people annually.
- Mammary cancer stem cells (MCSCs) promote aggressive tumors with extensive vascularization and are inherently resistant to most available therapies, thereby resulting in increased incidence of relapse and recurrence.
- Therefore, there remains a critical need to identify treatments effective against MCSCs.

Health disparities exist in breast cancer outcomes

- This complex disease involves many heterogeneous cell types. High contents of MCSCs are found in higher grade, undifferentiated triple negative breast cancers, which are frequently diagnosed in pre-menopausal African American (AA) women.
- AA women tend to suffer worse outcomes to chemotherapy, compared to Caucasian women.
Goals of Partnership

1) Develop assays in a nude mouse model system to report the effects of VA and other drugs on its intended target or a surrogate marker of efficacy

2) Apply relevant assays developed endogenously or received from investigators outside of the Translational Core Laboratory to a variety of tumor models in vitro and/or in vivo

3) Continue to work as a team in seeking mainstream funding to establish a research resource for drug development and discovery

Materials/Methods

Develop assays in a nude mouse model system to report the effects of VA and other drugs on its intended target or a surrogate marker of efficacy

1) Enriched for MCSCs from breast cancer cell lines and human tumors by cell sorting

2) Propagated them as mammospheres in vitro and in vivo as xenografts

3) Formed transplantable tumors from MCSCs in immunocompromised nude mice

4) Treated with aqueous extracts of Vernonia amygdalina

5) Determined its effect on proliferation of MCSCs and level of pERK1/2 expression

Enriched for MCSCs from breast cancer cell lines and human tumors by cell sorting

- Tumors were dissociated with 0.2% collagenase (1-2 hrs) and normal mammary tissues were subjected to hyaluronidase/collagenase treatment (8hrs). Single cells (50 cells/well) were plated in 24-well ultra-low attachment plates under defined mammosphere conditions.

- Cells from breast cancer cell lines were plated under well defined low attachment mammosphere conditions.

- Spheres were allowed to grow for 12 days and enrichment of MCSCs in mammospheres were determined by staining for high CD44+ and ALDH1+ or low CD24+ expressions.
Propagation of MCSCs as mammospheres in vitro and in vivo as xenografts

MCSCs/MSCs from human mammary epithelial cell lines MCF10A and MCF10A^RAS, and cells from human tumors (from fresh core biopsies of triple negative breast tumors from Cooperative Human Tissue Network and National Disease Research Interchange) were:

- Induced to differentiate into tubular and epithelial-like structures (exposure to low concentrations of lenti-virus expressing eGFP)
- Grown at clonal dilutions under sphere-forming conditions

Formation of transplantable tumors from MCSCs in immunocompromised nude mice

- Cells in the mammospheres were counted and 100,000 cells/mammary fat pad were injected into nude mice.
- Xenographs were formed in 4-6 weeks in nude mice.

Results

Mammospheres had self renewal capability. They could be dissociated and passaged multiple times, both in vitro as spheres and in vivo as xenografts.
Fractionation Studies and Treatment of Mammospheres with VA

- Cell fractionation experiments were done to determine the nuclear to cytosolic ratio of pERK 1/2 in MCF10A^RAS mammospheres.
- MCF10A^RAS cells were grown under mammospheres conditions for 6 days after which they were:
  1) Treated with various concentrations of VA for 48h
  2) The spheres were collected, fixed and stained for pERK1/2 and cMyc.
  3) Immunofluorescence analysis of these mammospheres was done.

Results - Cont’d
There are elevated levels of nuclear pERK1/2 compared to cytosolic levels in mammospheres.

Results - Cont’d
Treatment with VA decreased MCSC proliferation and mammosphere initiating ability in vitro, and reduced the levels of pERK1/2 and c-Myc.
Methods and Material (con’d)

Comparison of VA to PD

Grown for 6 days after which they were plated on high attachment plates in presence of high serum and simultaneously treated with various concentrations of VA or PD. After 48h of treatment the cells were fixed and analyzed.

Results- Cont’d

VA was more effective than PD98059 in inducing differentiation of MCSCs, and treatment with VA or PD98059 delayed formation of xenographs (due to injection of MCSCs into nude mice) from 4-6 weeks to 12 weeks.

Future Studies

Based upon these pilot findings, we propose to conduct studies to determine whether Veronica amygdalina (VA) treatment will reduce the initiation and progression of MCSC-induced xenografts in vivo (in nude mice).
Objectives

- Evaluate the therapeutic efficacy of pretreatment with *Vernonia amygdalina* in reduction in the number of MCSC-induced xenografts *in vivo*
- Assess the ability of *Vernonia amygdalina* to reduce aggressiveness of established xenografts in nude mice

Future Studies (cont’d)

- Determine the effects of combined treatment regimens of VA with other agents such as Tamoxifen, Taxol, Vinristine, Carboplatin, or other chemotherapeutic drugs
- Pre-treat nude mice with pristane, which will increase the efficiency of tumor formation
- JSU will extend the partnership with CDU in the form of a Memorandum of Understanding
- Dr. Pervin will supervise the training and eminent upgrade of the current Animal Facility at Jackson State University (JSU)
- She will also train Dr. Carolyn Howard on tumor transplantation and analysis so that future work relating to immune-compromised animal models could be carried out at JSU

Conclusion

The primary positive impact of our findings would be evidence-based scientific verification of a natural product that could be considered for the prevention and treatment of triple negative breast cancer in humans.

It is our expectation that this work will translate from mice to eventual VA clinical trials, as well as a model to evaluate other therapeutic agents for use in humans.

It is likely that the inhibitory effect of VA toward MSCs directly involves inhibition of the MAP Kinase activation pathway.
Acknowledgments

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RCMI 13th International Symposium Organizers

Questions

MICHAEL PEREZ
University of Hawaii at Manoa
The purpose of this investigation is to determine if a relationship exists between the administration of dietary supplements containing 5-ALA and sleep.
In a previous study conducted to examine the relationship between 5-ALA and pre-diabetes, a questionnaire covering a wide range of measures of health was administered. The study placed participants on the 5-ALA supplement for a period of 12 Weeks. In the course of this study, some interesting results relating to sleep patterns emerged.

Here is a summary of some of the survey results pertaining to sleep in some of the participants:

<table>
<thead>
<tr>
<th>Case #</th>
<th>Sleep at Week 0 (first day to start 5-ALA)</th>
<th>Sleep at Week 12 (with 12 wks 5-ALA intake)</th>
<th>Sleep at Week 16 (4 wks after 5-ALA ended)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 4</td>
<td>6.5 hours, wakes up several times</td>
<td>7.25 hours, mostly continuous sleep</td>
<td>Pattern reverting to waking up several times at night &amp; less restful sleep.</td>
</tr>
<tr>
<td>Case 13</td>
<td>8.0 hours sleep and does not take a &quot;nap&quot; during the day</td>
<td>7.5 hours, able to take a 10 min. &quot;nap&quot; during the day; now reports having &quot;Normal&quot; energy.</td>
<td>Feels &quot;tired&quot;, &quot;no energy&quot;, &quot;sleepy&quot;.</td>
</tr>
</tbody>
</table>

These data support the hypothesis that 5-ALA may be related to improved sleep. The fact that sleep patterns improved while on the 50 mg supplement, and then returned to previous patterns when the supplement was stopped, provide strong rationale for this pilot investigation.
5-Aminolevulinic Acid (5-ALA)

5-ALA is a delta amino acid (Non-Protein).
It is found in many common foods:
- Spinach
- Tomatoes
- Mushrooms
- Potatoes
- Squid
- Ground beef
- Wine
- Soy sauce

The normal intake from food containing 5-ALA is 1–2 mg/day.

5-ALA is synthesized by the body at a rate of 600 mg/day.

After 40 years of age, the body begins to produce about 550 mg/day.

5-ALA is the first compound in the porphyrin synthesis pathway.
This is the pathway that leads to heme production in mammals.

Animal research has shown that administration of 5-ALA can directly enhance aerobic energy metabolism.

*Rodriguez BL, Curb JD, Davis J, Shintani T, Perez M, Alina P, 

Increased level of glucose in the cell results in decreased 5-ALA production.

Decreased heme production occurs during aging; at age 40 the human body produces 50 mg/day less.

This results in decreased hemoglobin production with decreased heme enzyme activity.

A decline in the mitochondrial electron transfer system (the primary cellular energy producer) follows, with decreased basal metabolism— as well as physical energy decline.

Melatonin is a chemical that occurs naturally in the brain.

Melatonin allows a person to become sleepy.

Melatonin is naturally produced during the day-shift phase, or phase-delay (Fig. 1)

Figure 1

Adapted from Lewy et al. and Khalsa et al. with permission.
The human body produces its own endogenous melatonin starting about two hours before bedtime, provided that the lighting is dim. This is known as Dim-Light Melatonin Onset, DLMO. This stimulates the phase-advance portion of the phase response curves and helps keep the body on a regular sleep-wake schedule.

The human body has a master circadian clock in a control center of the brain known as the suprachiasmatic nucleus (SCN). The SCN located in the anterior hypothalamus is the body’s generator of the circadian rhythms. This cycle controls sleep and wakefulness. In order for this system to function efficiently, it needs information from a variety of sources, which include physical activity, social activities and to experience day and night.

The ganglion cells in the retina collect light information for the SCN. These cells produce a pigment called melanopsin and are particularly sensitive to light. The Pupillary Light Reflex regulates circadian rhythms. Light exposure is needed by the pineal gland to produce melatonin.
Since the day-phase contributes to melatonin production.

Melatonin allows a person to be sleepy.

Extended or enhanced day-phase activity could result in increased melatonin during the evening.

Hypothesis: 5-ALA could, in fact, enhance day-phase activity, which could inversely allow a person to experience a better sleep cycle.

The Theory of 5-ALA and Sleep

Design. This is a double-blind, randomized parallel-group comparison study.

Sample. 40 participants between ages 40 and 70 were randomized to the following 2 study groups:
- Control Group - 20 participants
- Intervention Group - 20 participants.

THE SUPPLEMENT SLEEP STUDY

Sleep Study DESIGN and SAMPLE

- Control Group - 20 participants
- Intervention Group - 20 participants.

Hypothesis: 5-ALA could, in fact, enhance day-phase activity, which could inversely allow a person to experience a better sleep cycle.
Inclusion Criteria
Both males and females will be equally recruited. Participants between 40 and 70 years who by self-report have insomnia or difficulty sleeping.

Exclusion Criteria
Those at a body weight of <110 or >250 lbs
Those with a history of porphyria, as S-ALA may cause adverse effects on porphyria patients.
Those with a history of hemochromatosis will also be excluded, as Sodium Ferrous Citrate (SFC) may cause adverse effects on hemochromatosis patients. Those with defects in porphyrin metabolism.
Those with a history of hepatitis, as SFC may cause an allergic reaction in this population.
Those with active liver disease and iron sensitivity.
Women who are pregnant, breastfeeding, and those participating in another clinical study will not be excluded.
Those with Ferritin levels elevated above 125% of normal on screening.

Study Schedule
- During Visit 5 (Week 10), participants were no longer taking the supplement.

Pittsburgh Insomnia Rating Scale (PIRS-20)

The PIRS-20 is copyrighted by the University of Pittsburgh*.

PIRS-20

- Items must occur in their original sequence, as this aspect is a deliberate design feature.
- The PIRS-20 has repeated measures of validity.
- The PIRS-20 is available as an open resource.
- A lower score indicates better sleep.
- Scale: 0 (good)-60 (bad)

RESULTS

Descriptive Statistics

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>52.15</td>
<td>7.41</td>
</tr>
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<td>7.41</td>
</tr>
</tbody>
</table>

N=40 Control 50 mg

Mean SD Mean SD

Age 54.75 7.91 56.3 6.17

N=20 50 mg % Mean Age

Asian/Filipino 50% 57.9
Native Hawaiian 20% 53

N=20 Control % Mean Age

Asian/Filipino 45% 53.8
Native Hawaiian 10% 62

Descriptive Statistics

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Mean SD Mean SD

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Asian/Filipino 50% 57.9
Native Hawaiian 20% 53

N=20 Control % Mean Age

Asian/Filipino 45% 53.8
Native Hawaiian 10% 62
The placebo effect is demonstrated on visits 2 to 3. Visits 3 to 5 show no response in either direction.
**PIRS-20 Score Decline**

- The PIRS-20 score declined on visits 2, 3, and 4.
- Visit 5, when no longer taking the supplement, the response begins to return to baseline.
- This is the expected outcome.

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**Results Table**

<table>
<thead>
<tr>
<th></th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
<th>Visit 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>22.45</td>
<td>19.05</td>
<td>19.95</td>
<td>19.95</td>
</tr>
<tr>
<td>5-ALA 50mg</td>
<td>29.95</td>
<td>21.10</td>
<td>16.10</td>
<td>20.65</td>
</tr>
</tbody>
</table>

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**Improvement in Sleep**

- Improvement in sleep in the group taking 50mg 5-ALA compared to control was significant.

**Null Model Likelihood Ratio Test**

- The rate of change per visit from visit 2 through 4 was -5.67 units on the sleep scale less than the control group with a *p* value of .0008.
- The rate of change from visit 4 to 5 when the participant was no longer taking the supplement was 4.55 units higher than the control with a *p* value of .0616, which is of borderline significance.
CONCLUSION

- There appears to be a relationship between the administration of dietary supplements containing 5-ALA and sleep.
- The results of this study suggest that 5-ALA does in fact improve sleep.
- The mechanism for what causes this still need to be explored.
- Additional Research is needed.

5-ALA Publications

- Perez M, Shintani T, Rodriguez B, Johnson C, Harrigan R. Role of 5-Aminolevulinic Acid and Sleep: An Integrative Review of The Literature (In Review)
- Perez M, Rodriguez B, Shintani T, Davis J, Johnson C, Harrigan R S-Aminolevulinic Acid (5-ALA): Analysis of Preclinical and Safety Literature (In Progress)

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“RCMI Multidisciplinary And Translational Research Infrastructure Extension, (RMATRIX)”

NIMHD
National Institute on Minority Health and Health Disparities

SBI Pharma
About This Presentation

- What would you do if your research sample does not exhibit reproducible biological activity?
- We encountered such a problem when we were studying immunostimulatory herbal medicine.
- Further studies revealed an unexpected mechanism by which the potency of the sample is regulated.
Oriental Herbal Medicine

- Herbal mixtures which collectively exhibit desired therapeutic effects
- Long-tested safety and efficacy (> 2000 yrs)
  - Now used by >80% of Japanese MDs

Juzen-taiho-to (JTT)

- A formulation to boost the immune system
- Used clinically in Japan for cancer patients undergoing chemo and radiation

Wolfiporia extensa (fungus)
Cnidium officinale (stem)
Angelica sinensis (root)
Atractylodis macrocephalae (stem)
Paeonia lactiflora (root)
Cinnamomum aromaticum (cortex)
Astragalus membranaceus (root)
Rehmannia glutinosa (root)
Codonopsis pilosula (root)
Glycyrrhiza uralensis (root)

JTT Stimulates Monocytes/Macrophages

- Model: tail vein injection of colon cancer
- JTT significantly reduced liver metastasis
- No effect when the monocyte/macrophage population was depleted by 2-chloroadenosine
- Active compounds not clear

Assays to Detect Monocyte Stimulation

- **ICAM-1**: Intercellular adhesion molecule (CD54)
  - Good biomarker of monocyte-stimulation
  - THP-1 cells (monocytic cell line) was used for our screening of JTT

Search for Monocyte-stimulatory Factor

- The fraction is a lipid mixture enriched with phytosterol glycosides
- The potency appeared to depend on HPLC solvents.
Activity Depends on Solvent Pre-treatment

- **Active Fraction**:
  - 90% MeOH aq
  - 100% MeOH

- **Dry & Dissolve in DMSO**
  - "90%" Sample
  - "100%" Sample

ICAM1 qRT-PCR

Hypothesis

- **Hypothesis**: The activity difference is caused by some difference in molecular assembly.

- To test this hypothesis, the following measurements were carried out for the two samples:
  - Atomic Force Microscopy
  - Dynamic Light Scattering

Atomic Force Microscopy

"90%" "100%"
Dynamic Light Scattering

ICAM1 qRT-PCR

“90%” “100%”

Freq
0 0.6
10 100 1000
D (nm)

What Does the Finding Suggest?

- New strategies to stimulate monocytes
- Oriental herbal medicine: Sophisticated mixtures of bioactive ingredients & formulation agents
- Molecular assembly was an important factor when evaluating biological activity of small molecules

Conclusion

- We purified a monocyte-stimulatory factor from JTT.
- Preliminary characterization of this factor suggested that nanoparticle-formation is important for the activity.
- Further characterization is underway to understand the mechanism of safe and effective immunostimulation as exhibited by JTT.
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Mr. Joon Kim (qRT-PCR)

Hunter College Gene Center

Prof. Hiroshi Matsui
Prof. C. Michael Drain
Dr. Roberto de la Rica
Dr. Gabriela Smeureanu

Abstract # 06.01.02

“Vitamin D Boosts Muscle Differentiation by Modulating Angiogenesis”

Jorge N. Artaza1,2, Leah A. Garcia1, Monica G. Ferrini1,2, Keith C. Norris1,2
1Department of Internal Medicine, Charles R. Drew University, 2David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.
Background I

- Hypovitaminosis D is highly prevalent in U.S. minority populations. A better understanding of its mechanisms of action could have important health disparity implications.

- Vitamin D (VD) is mostly known by its role in the regulation of calcium homeostasis and bone metabolism.

- Increasing evidence indicates that VD plays an essential role in many other tissues, including skeletal muscle, especially in relation to muscle weakness and muscle pain.

- Cross-sectional studies in community-dwelling older adults have found a direct association between VD status and parameters of physical performance.

Background II

- Mice lacking the VDR show a skeletal muscle phenotype with smaller and variable muscle fibers & persistence of immature muscle gene expression during adult life.

- We recently demonstrated that VD exerts a pro-myogenic effect on muscle cells (1); this has prompted our investigation of VD's effects on angiogenesis, a vital process for new capillary development and tissue repair.

- Angiogenesis is the process of new capillary development and growth and is a vital process during the adult life span for regeneration of damaged tissues.

- In addition to myogenic differentiation, regenerative therapies for skeletal muscle injuries also need to consider the promotion of revascularization, as a consequence of tissue scarring.

Specific Aims

The aim of the present study is:

- To test whether 1,25-D3—the active form of vitamin D—in addition to promoting myogenic differentiation can also modulate the expression of key angiogenic growth factors and angiogenic growth factor inhibitors that may ultimately facilitate muscle regeneration and repair.
Hypothesis

1,25-D3—the active form of Vitamin D—will modulate the expression of angiogenesis growth factors and inhibitors, which may be an additional mechanism by which Vitamin D promotes myogenesis.

Background III: Vitamin D Metabolism

Vitamin D3 precursors are synthesized and stored in skin. Vitamin D is converted to 25(OH)D by UV light. 25(OH)D is then converted to 1,25(OH)2D by 25-OHase and α-OHase. Deficiency of Vitamin D (<20 ng/ml) leads to 400-2,000 IU/day intake.

Material & Methods: C2C12 Myoblasts

The C2C12 Myoblasts were propagated for a few days and incubated with 100 nmol/L of 1,25(OH)2D3 with Ethanol < 1% as vehicle or placebo.

End-points:

a) Expression of Angiogenic Growth Factors and Angiogenic Growth Factors Inhibitors were analyzed by:
- PCR arrays
- Real Time PCR
- Proteome Array analysis
- Western Blts
Results

25-D3 Induces the Expression of VDR in C2C12 Myoblasts

Differential Expression of Angiogenic Growth Factors and Inhibitors in 1,25-D3 Treated and Untreated Muscle Cells

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Description</th>
<th>Reference Sequence</th>
<th>24 hours</th>
<th>4 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fgf-1</td>
<td>Fibroblast growth factor 1</td>
<td>NM_008010</td>
<td>+1.03</td>
<td>+5.77b</td>
<td>+11.47c</td>
</tr>
<tr>
<td>Vegf</td>
<td>Vascular endothelial growth factor A</td>
<td>NM_009505</td>
<td>+3.28c</td>
<td>+6.55b</td>
<td>-1.09</td>
</tr>
<tr>
<td>Timp-3</td>
<td>Tissue inhibitor of metalloproteinase 3</td>
<td>NM_011595</td>
<td>-2.47c</td>
<td>-3.74b</td>
<td>-1.10</td>
</tr>
<tr>
<td>Fgf-2</td>
<td>Fibroblast growth factor 2</td>
<td>NM_008006</td>
<td>-2.13c</td>
<td>-2.06b</td>
<td>-1.87a</td>
</tr>
<tr>
<td>Fgf-6</td>
<td>Fibroblast growth factor 6</td>
<td>NM_010204</td>
<td>+1.13</td>
<td>+1.35</td>
<td>+1.13</td>
</tr>
</tbody>
</table>

Total RNA from cells treated with or without 1,25-D3 for 24 hours, 4 days and 10 days was subjected to RT real time PCR by the Angiogenic Growth Factor and Angiogenesis Growth Factor Inhibitor arrays, and the ratios between the treated 1,25-D3-treated and 1,25-D3-untreated cells were corrected by GAPDH, Hprt1, Hsp90ab1, and Actb were calculated for assays performed in triplicate. Experiments were performed in triplicate.

P < 0.05 a
P < 0.01 b
P < 0.001 c
Changes in Angiogenic Protein Expression Profile after 24 hours of Continuous Incubation with 1,25-D3

Changes in Angiogenic Protein Expression Profile after 4 days of Continuous Incubation with 1,25-D3

mRNA & Protein Up-regulation of FGF-1 Expression Upon Incubation of Muscle Cells with 1,25-D3
mRNA & Protein Up-regulation of VEGFα Expression Upon Incubation of Muscle Cells with 1,25-D3

- **24 hours**
  - Control-1
  - Control-2
  - VD-1
  - VD-2
  - 1,25-D3 (100nM)
- **4 days**
  - Control-1
  - Control-2
  - VD-1
  - VD-2
  - 1,25-D3 (100nM)

**Western blot**
- VEGFα
- 40kDa
- 42 kDa
- GAPDH

mRNA & Protein Down-regulation of FGF-2 Expression Upon Incubation of Muscle Cells with 1,25-D3

- **24 hours**
  - Control-1
  - Control-2
  - VD-1
  - VD-2
  - 1,25-D3 (100nM)
- **4 days**
  - Control-1
  - Control-2
  - VD-1
  - VD-2
  - 1,25-D3 (100nM)

**Western blot**
- FGF-2
- 31 kDa
- 40kDa
- GAPDH

mRNA & Protein Down-regulation of TIMP-3 Expression Upon Incubation of Muscle Cells with 1,25-D3

- **24 hours**
  - Control-1
  - Control-2
  - VD-1
  - VD-2
  - 1,25-D3 (100nM)
- **4 days**
  - Control-1
  - Control-2
  - VD-1
  - VD-2
  - 1,25-D3 (100nM)
Vitamin D Role on Myogenic cell Differentiation

Summary of Results

- In addition to our previous findings (1), which demonstrated that supplementing C2C12 myoblasts with 1,25-D3 promotes myogenesis through:
  - Translocation of VDR to the nucleus.
  - Increasing the expression of IGF-2 and Follistatin.
  - Decreasing the expression of IGF-1 and Myostatin (a negative regulator of muscle mass).

We have also found that addition of 1,25-D3 induces:
- Increased expression of VEGF and FGF-1, two well described pro-angiogenic growth factors that promotes neo-vascularization, tissue regeneration and myogenesis.
- Decreased expression of IGF-2 and TIMP-3, two main angiogenic / myogenic inhibitors, which both have been described to promote myogenic inhibition through FGF-2's interaction with IGFs.

Conclusions

- These results extend our previous findings and demonstrate the modulation of angiogenesis by Vitamin D, which may be an additional mechanism by which VD promotes myogenesis.
- This study supports the mechanistic rationale for the administration of Vitamin D and/or Vitamin D analogs to treat select muscle disorders and may also provide an alternative solution for therapies that directly manipulate VEGF and FGF's to promote angiogenesis.
- These basic mechanisms could have important implications for the progression of chronic diseases, many of which are disproportionately prevalent in minority populations.
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- Monica G. Ferrini, M.S., PhD
- Keith C. Norris, M.D.

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THANK YOU!!

Q&A
Thank you for participating!