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AYUR-INFORMATICS: BLOCKING THE TRANSLATION PATHWAY OF FUMARATE HYDRATASE MUTATION GENE BY RNAI TECHNIQUE AND ESTABLISHING AN AYURVEDIC REMEDY FOR UTERINE FIBROIDS

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ABSTRACT

Fumarate hydratase precursor (mutation) protein of *Homo sapiens* was taken. Homology modeling studies were done and 3D structure of FH protein was modelled. Ayurvedic herbs *Aloe vera, Commiphora mukul, Asparagus racemosa* and *Saraca indica*'s active components were selected, combined & docked with Fumarate hydratase mutation protein. The docking proves that the combination is effective in curing uterine fibroids. Again, by means of RNAi technique, the translation–pathway of a Fumarate Hydratase mutation gene is blocked.

Keywords- Uterine fibroids; Mutation; Homology modeling; Computer Aided Drug Designing; Ayurveda; *Aloe vera*; *Commiphora mukul*; *Asparagus racemosa*; *Saraca indica*; RNAi technique.

INTRODUCTION

Fibroids are tumors that grow in the uterus (womb). They are benign, of non-cancerous origin, and are made up of muscle fibre. They are rare in women under the age of 20, most common in women in their 30s and 40s, and tend to shrink after the menopause. If a fibroid or cluster of fibroids is particularly large or is growing on the outside of the uterine wall, it can sometimes push the uterus aside or force it to grow abnormally. A uterine growth may also put pressure on the bladder or intestines. Fibroid tumors are solid tumors which are made of fibrous tissue, hence the name 'fibroid' tumor. Most often fibroids occur as multiple tumor masses which are slow-growing and often cause no symptoms. African American woman are at three- to five-times greater risk than white women for fibroids. Smoking and oral contraceptive exposure have been inconsistently reported as risk factors [1].

Fumarate hydratase mutation protein is the protein involved in causing the disease. Fumarate hydratase (or Fumarase) is an enzyme that catalyzes the reversible hydration/dehydration of Fumarate to S-malate [1].

Fumarate hydratase locus is on chromosome 1, possibly in the area 1q42. The Multiple Leiomyoma Consortium identified heterozygous germline mutations of FH in patients with multiple cutaneous and uterine leiomyomas. Female FH mutation carriers are also at high risk of early-onset uterine fibroids that frequently require hysterectomy. The cutaneous, uterine, and renal manifestations were described as a single entity by and the condition was renamed hereditary leiomyomatosis and renal cell carcinoma (HLRCC) [2].

Germline mutations in the FH gene, encoding fumarate hydratase, an enzyme that catalyzes the conversion of fumarate to malate in the Krebs cycle, predispose to dominantly inherited uterine fibroids, skin leiomyoma, and papillary type II renal cell cancer**.** These mutations are predicted to result in absent or truncated protein or in substitutions or deletions of highly conserved amino acids. In HLRCC, the most common clinical features are leiomyomas of the skin and uterus, and in a subset of the families, renal cell cancer (RCC) and uterine leiomyosarcoma (ULMS) occur frequently at young age [3, 4].

Ayurvĕda is the Indian traditional system of medicine. Different types of plant parts are used for the Ayurvedic formulation. In India most of them, where Ayurvedic treatment is frequently used, provides instructions to local people how to prepare medicine from the herbs.

Aloes compound from *Aloe vera* was found to be beneficial in cases of functional sterility and disturbed menstrual function. Various constituents of *Aloe vera* have been shown to have anti inflammatory activity in uterine disorders. Aloes are bitter thus helps to balance the glucose level of the blood and its zinc and other mineral content stimulate production of insulin by the pancreas. When ingested, it helps to lower cholesterol, reduces inflammation resulting from radiation therapy, increases blood vessel regeneration in lower extremities of people with poor circulation, soothes stomach irritation, aids healing, and acts as a laxative. Aloes also increase blood flow to the uterus thus can be used to induce menstrual flow and the remedy for uterine leiomyomas [5].

Commiphora mukul can be used in painful urination, pus discharge in urine, kidney stones, in reproductive system, dysmenorrhoea, infertility, uterine leiomyomas, and anti inflammatory. As an antagonist it is used for increased level of oestrogen. Since increased level of oestrogen level causes the uterine leiomyoma, it is used [6].

Asparagus Racemosa promotes the health of the reproductive organs. It is beneficial in providng nourishment to tissues weakened by menstrual disorders. Mainly used as the ayurvedic rejuvenation for women. It purifies the blood and has an harmonizing effect on the hormonal system. The bark has a stimulating effect on the endometrium and ovarian tissue, is useful in menorrhagia due to uterine fibroids, in

leucorrhea and in internal bleeding , where ergot is indicated. It is well established for its effectiveness in menorrhagia and dysmenorrheal. It is also has a stimulatory effect on the ovarian tissue and may produce an oestrogen-like effect that enhances the repair of the endometrium and stops bleeding [7, 8].

The bark of the tree, *Saraca asoca,* is effective for excessive blood loss during menstruation due to the presence of uterine fibroids, leucorrhoea and other causes. Ashoka happens to be a uterine stimulant and increases uterine contractions. It is a known herb to help female health. The bark has a stimulating effect on the endometrium and ovarian tissue, is useful in menorrhagia due to uterine fibroids, in leucorrhea and in internal bleeding, where ergot is indicated. It is also has a stimulatory effect on the ovarian tissue and may produce an oestrogen-like effect that enhances the repair of the endometrium and stops bleeding. The bark of the tree is effective for excessive blood loss during menstruation due to the presence of uterine fibroids, leucorrhoea and other causes. Ashoka happens to be a uterine stimulant and increases uterine contractions [4].

The RNAi technology is gaining its importance in the field of Molecular Biotechnology/Bioinformatics. RNA interference (RNAi) is a system within living cells that helps to control which genes are active and how active they are. RNAs are the direct products of genes, and these small RNAs can bind to specific other RNAs and either increase or decrease their activity, for example by preventing a messenger RNA from producing a protein. RNA interference has an important role in defending cells against parasitic genes – viruses and transposons – but also in directing development as well as gene expression in general. The RNAi pathway is found in many eukaryotes including animals and is initiated by the enzyme Dicer, which cleaves long double-stranded RNA (dsRNA) molecules into short fragments of ~20 nucleotides. One of the two strands of each fragment, known as the *guide strand*, is then incorporated into the RNA-induced silencing complex (RISC). The selective and robust effect of RNAi on gene expression makes it a valuable research tool, both in cell culture and in living organisms because synthetic dsRNA introduced into cells can induce suppression of specific genes of interest. Exploitation of the pathway is also a promising tool in biotechnology and medicine. In 2006, Andrew Fire and Craig C. Mello shared the Nobel Prize in Physiology or Medicine for their work on RNA interference in the nematode worm *C. elegans*. which they published in 1998.

METHODOLOGY

Softwares/Web Servers used

1) modeller9v7 MODELLER (copyright © 1989-2008 Andrej Sali) http://salilab.org/index.html
2) Swiss-PdbViewer v4.01 by Nicolas Guex , Alexandre Diemand , Manuel C. Peitsch , & Torsten Schwede (Swiss Institute of Bioinformatics) http://spdbv.vital-it.ch/index.html
3) ACD/ChemSketch Freeware, version 11.00, Advanced Chemistry Development, Inc.,

Toronto, ON, Canada, www.acdlabs.com, 2008.
4) SAVES: Stuctural Analysis and Verification Server http://nihserver.mbi.ucla.edu/SAVES/
5) ArgusLab 4.0.1, Mark A. Thompson, Planaria Software LLC, Seattle, WA http://www.arguslab.com
6) HEX 5.1 University of Aberdeen, Scotland, UK http://www.csd.abdn.ac.uk/hex/
7) LigandScout Structural Bioinformatics group at Tel -Aviv University http://bioinfo3d.cs.tau.ac.il/PatchDock/index.html
8) Genamics Expression http://genamics.com/expression/index.htm
9) Microsynth http://www.microsynth.ch/sirnadesign/sirnadesign.html

With the help of homology modeling software, modeler, the 3d structure of FH mutation protein was modeled. The chemical structure of aloe-emodin, guggulsterone, shatavarin and 17-ketosterol were drawn using ACD/Chemsketch software and converted to *.pdb and then docked with FH mutation protein using HEX software.

The DNA sequence of FH mutation protein was converted to RNA sequence using Expression software. This RNA sequence was subjected RNAi treatment using Microsynth software.

RESULTS

The Fumarate Hydratase mutation protein, human FH was retrieved from UniProt database (accession number P07954) for this work. Homology modeling studies were done using modeler 9v7 software using templates (homologous proteins in the RCSB's pdb database). The template proteins taken were 1YFMA (identity-66%), 3GTDA (identity-62%), 1FUOA (identity-60%) and five models (3D structures) of the FH mutation protein were generated. The models generated by Modeller were analyzed by SAVES server and the best stable model is selected (Fig 1).

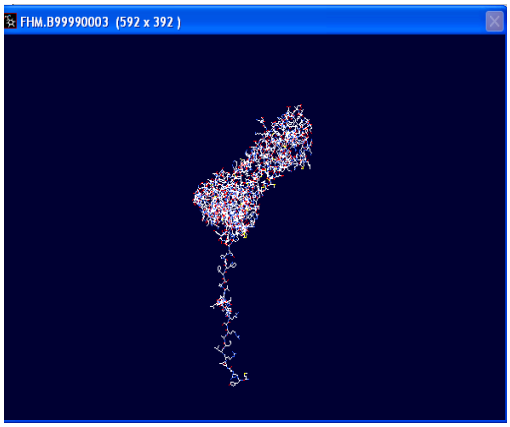

Fig 1-3D Structure of FH mutation protein (visualization in SPDBV)

For proposed treatment, known principal alkaloids from *Aloe vera*, *Commiphora mukul*, *Asparagus racemosa* and *Saraca indica* namely aloe-emodin, guggulsterone, shatavarin and 17-ketosterol respectively were selected.

The chemical structure of aloe-emodin (fig 2), guggulsterone (fig 3), shatavarin (fig 4) and 17-ketosterol (fig 5) were drawn using ACD/Chemsketch software. Again, the structures of aloeemodin, guggulsterone, shatavarin and 17-ketosterol were merged as one structure (fig 6) (proposed treatment). This combination is converted to 3d which was converted to *.pdb structure (fig 7) and submitted to HEX server along with FH mutation protein for docking.

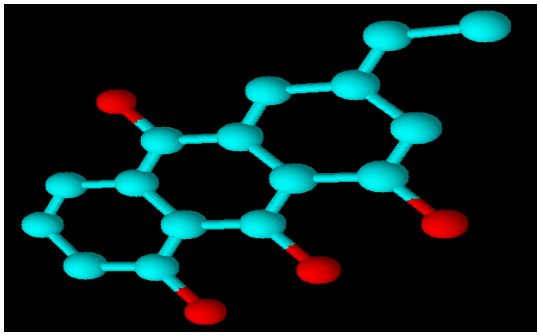

Fig 2-Chemical structure of aloe-emodin
in chem3d viewer software

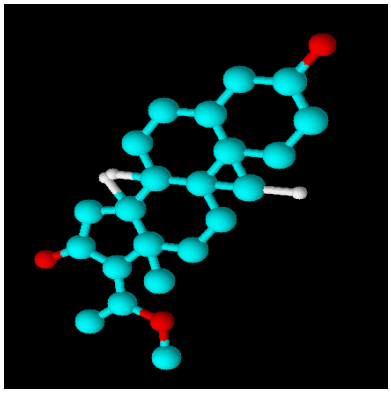

Fig 3- Chemical structure of guggulsterone
in chem3d viewer software

Fig 4- Chemical structure of shatavarin
in chem3d viewer software

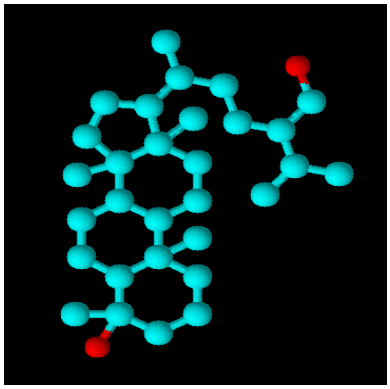

Fig 5 - Chemical structure of 17 ketosterol
in chem3d viewer software

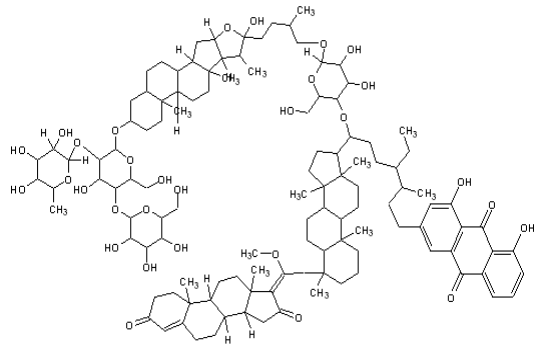

Fig 6- Combined Structure of aloe-emodin,
guggulsterone, shatavarin and 17-ketosterol

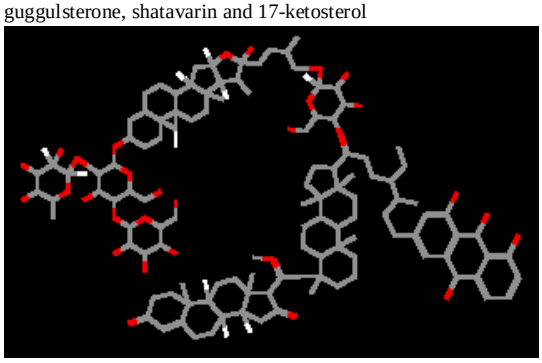

Fig 7 - Combined Structure of aloe-emodin, guggulsterone, shatavarin and 17-ketosterol (visualization in Arguslab software)

The FH.pdb protein obtained after homology modeling was analyzed with SAVES server. The results obtained were:

Model 1

Residues in most favoured regions [A,B,L] 407 92.9%

Residues in additional allowed regions [a,b,l,p] 27 6.2%

Residues in generously allowed regions [~a,~b,~l,~p] 4 0.9%
Residues in disallowed regions 0 0.0%

Model 2

Residues in most favoured regions [A,B,L] 407 92.9%
Residues in additional allowed regions [a,b,l,p] 29 6.6%
Residues in generously allowed regions [~a,~b,~l,~p] 2 0.5%
Residues in disallowed regions 0 0.0%

Model 3

Residues in most favoured regions [A,B,L] 412 94.1%
Residues in additional allowed regions [a,b,l,p] 24 5.5%
Residues in generously allowed regions [~a,~b,~l,~p] 2 0.5%
Residues in disallowed regions 0 0.0%

Model 4

Residues in most favoured regions [A,B,L] 399 91.1%
Residues in additional allowed regions [a,b,l,p] 35 8.0%
Residues in generously allowed regions [~a,~b,~l,~p] 4 0.9%
Residues in disallowed regions 0 0.0%

Model 5

Residues in most favoured regions [A,B,L] 399 91.1%
Residues in additional allowed regions [a,b,l,p] 34 7.8%
Residues in generously allowed regions [~a,~b,~l,~p] 5 1.1%
Residues in disallowed regions 0 0.0%

As per the output of the SAVES server, model 3 was judged as the best protein because it has maximum residues in the most favoured region. The Ramachandran plot of the model is given below (fig 8):

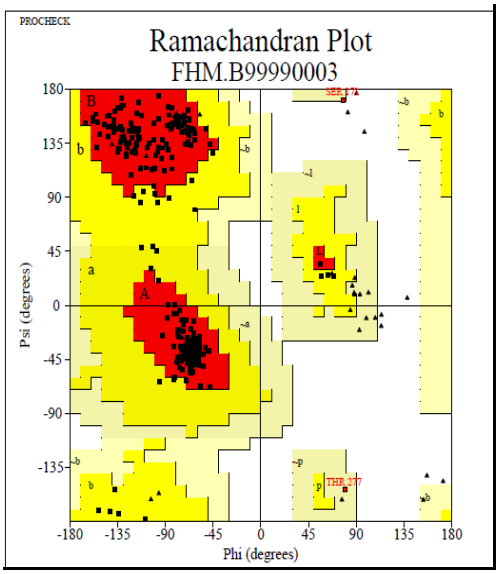

Fig 8- Ramachandran Plot analysis FH.pdb (model 3)

This model (#3) was docked with combined structure of aloe-emodin, guggulsterone, shatavarin and 17-ketosterol (in pdb file format). Below is the docking result visualized in LigandScout software (the combined structure of aloe-emodin, guggulsterone, shatavarin and 17-ketosterol is seen within light green box) (fig 8). Docking score 12506 given by PATCHDOCK server.

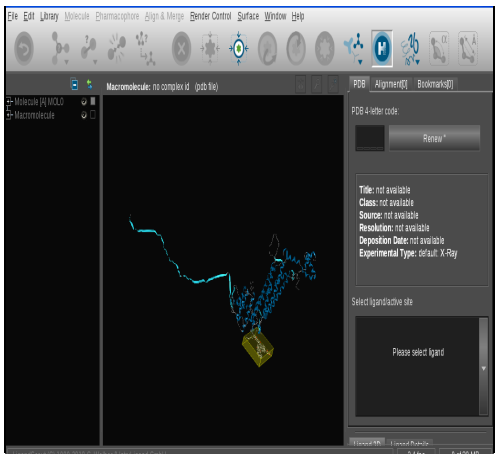

Fig 8- Docked structure (visualization in LigandScout)

The corresponding nucleotide sequence of the FH mutation protein was retrieved (AAH17444) and was submitted to Genamic Expression software for getting

the corresponding RNA sequence (fig 9). This retrieved RNA sequence was submitted to Microsynth server for breaking the sequence (fig 10). Since the RNA sequence is broken down, it cannot be joined back and converted to protein, thus the translation pathway is blocked.

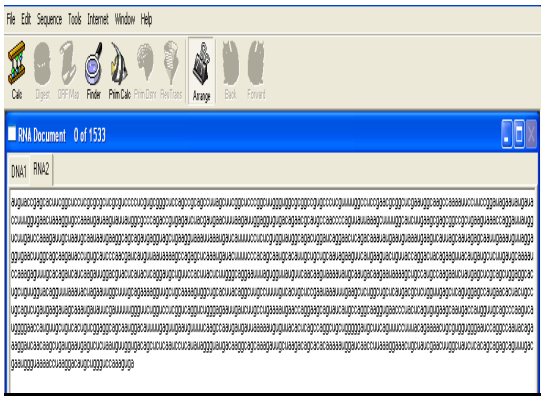

Fig 9 - Converson Of DNA To RNA Using Genamics Expression

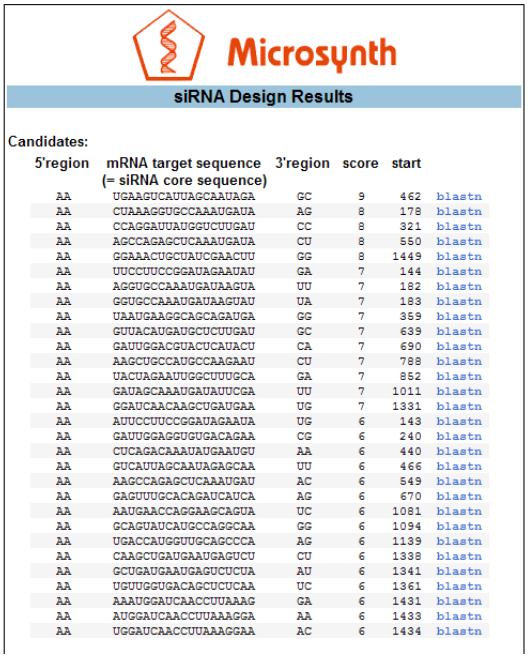

Fig 10 – Slicing of RNA by Mycrosynth server

CONCLUSION AND DISCUSSION

The present study was based on the disease Uterine Fibroids which is a non-cancerous tumor of the myometrium of uterus. It is found out, that the ligand (combination of four compounds (Aloe emodin, Shatavarin, Guggulsterone, 17-ketosterol) docks with the protein (Fumarate Hydratase mutation) with docking score 12506. Also by means of RNAi technique, the translation–pathway of a Fumarate Hydratase mutation gene is blocked.

This *in-silico-ayurvedic* work highlights two important landmark achievements towards this disorder: (i) mutation as the cause of Uterine Fibroids and identification of the mutant gene & its corresponding protein, and (ii) application of Indian ayurvedic herbs

towards treatment/cure of this disorder. These achievements should prove to be a boon to those women who are sufferers of this particular disorder.

Since the above work is an *in-silico* work, the compound combination (Aloe emdin, Shatavarin, Guggulsterone, 17-Ketosterol) has to go to clinical trials to establish its efficacy. Again it is found that *(Aloe vera, Asparagus racemosa, Commiphora mukul, Saraca indica)* is having anti-inflammatory and hormonal imbalance properties, hence, can be used to treat uterine fibroids. Also, *Saraca indica* plant has found importance in ayurvedic medication to cure all types of uterine disorders and it has been mentioned in ancient ayurvedic texts.

It is found in this study that RNAi technique used to block the translation pathway of Fumarate hydaratse mutation gene. Hence if this technique is commercialized, we can find a solution / cure for uterine fibroids.

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