

## ZAMZAM WATER GENE DOWNREGULATION IN UTERINE FIBROCHONDROSARCOMA CELL LINE

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### ABSTRACT

**Introduction:** Zamzam water is unique in its natural characteristics as zamzam water has a strong anti-inflammatory and it caused downregulation of genes after induction of colon tumor in rate, we test this hypothesis on human by studying the cell lines from uterine fibro-chondrosarcoma.

**Material and methods:** Uterine fibrochondrosarcoma cell line were incubated with zamzam water 20 c.c. for one week after previous culture in Dulbecco's modified eagle's medium. Gene expression was done microarray hybridization.

**Results:** Zamzam water causes downregulation genes which affect growth, integrin, insulin like growth factor and nuclear factor of kappa, and interleukin.

**Conclusion:** Zamzam water causes a down regulation of genes affecting growth of uterine fibrochondrosarcoma.

### INTRODUCTION

Zamzam water is unique in its natural characteristics; zamzam water has special optical parameters that are different than those of bottled drinking and distilled water. The aim of this work is to explore the oncolytic action of zamzam water i.e. its ability to reduce the size of tumor and its spread in human uterus. As zamzam water has strong anti-inflammatory and oncolytic action<sup>(1,2,3,4)</sup>.

### MATERIAL AND METHODS

Uterine fibrochondrosarcoma cell line was obtained from a grade I primary uterine fibrochondrosarcoma in 48 year old female with no history hormone treatment, any method of contraception she was para 8, no previous surgical operation, no medical disorder, no history of corticosteroid<sup>(5,6)</sup> this uterine fibrochondrosarcoma cell line

maintains the chondrocytic phenotype. It was cultured in Dulbecco's modified eagle's medium. Cells were washed with the culture medium and incubated with 20c.c zamzam water for one week total RNA was extracted as the manufacture's protocol<sup>(7)</sup>. Also, cDNA synthesis was performed by detection method (3DNA array 50) as per the manufacturers protocols). Then microarray hybridization<sup>(7,18, 20, 27)</sup>.

## RESULTS

We studied for the first time in literature the effect of zamzam water on uterine fibrochondrosarcoma cell line which was obtained from grade I primary uterine fibrochondrosarcoma incubated with 20 c.c. Zamzam water for one week the result of this work was summarized in the following table.

**Table (1): Zamzam water induced downregulation genes in uterine fibrochondrosarcoma cells line**

Gene name	Decrease
Caspase 6 , apoptosis –related cysteine protease integrin, beta 2 (antigen CD18 (p95), lymphocyte function- associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)	6.5
Caspase 2, apoptosis-related cysteine protease (neural precursor cell expressed, developmentally down-regulated 2)	6.2
Insulin –like growth factor binding protein 3	6.5
Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	11.5
Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)	3.9
Nuclear factor of kappa light polypeptide gene enhancer in b-cells 1 (p105)	15.8
Small inducible cytokine subfamily A (Cys –Cys), member 13	9.5
Tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)	15.1
Tumor necrosis factor ligand, supefamily, member 4	8

## DISCUSSION

Zamzam water is a miracle<sup>(1,2,3)</sup> one it's aspect is strong anti-inflammatory action, the anti-inflammatory action and the oncolytic action<sup>(4)</sup> is due to its flavenoid like action<sup>(9,26)</sup>, we have reported that zamzam water had strong anti tumor necrosis factor<sup>(4)</sup> TNF $\alpha$  and interleukin I (IL1)<sup>(6,19)</sup> knowing that chondrocytes<sup>(8)</sup> is the basic cell of the cartilage, so we choose the lesion in which chondrocytes in an active state in including growth and apoptosis i.e. uterine fibrochondrosarcoma cell line which was obtained from a grade I primary chondrosarcoma with no history of hormonal contraception and incubation with 20 c.c. zamzam water for one week and cell line was processed for cDNA, and microarray hybridization was carried<sup>(17,18,20,24,27)</sup>. We found that the

following genes were down regulated: tissue inhibitor of metalloproteinase I, integrin, cytokine subfamily member 13, tumor necrosis factor, caspase 6, telomerase, insulin like growth factor, nuclear factor of kappa, interleukin 12A, caspase 2 and insight to table (1) we found that zamzam water cause downregulation of tissue inhibitor of metalloproteinase state of activity of tissue inhibition of metalloproteinase<sup>(10,21)</sup> determine cartilage breakdown and resultant tissue destruction, metalloproteinase<sup>(11)</sup> were effective in resorption of cartilage<sup>(12,13,15)</sup>, again zamzam water cause down regulation of integrin alpha 5. Integrins function both as cell adhesion receptors and as intracellular signaling receptors<sup>(25)</sup>.

Also, zamzam water causes down regulation in insulin like growth factor binding protein (IGFBP-3)<sup>(23)</sup> which was reported to be increased in sever cartilage affection due to autocrine production, the nuclear factor of kappa gene<sup>(12,13,16)</sup> which was greatly decreased 15.8 fold meaning that it is the most affected gene. The least affected gene was interleukin 12A<sup>(22,25)</sup> 3.9 fold reduction so gathering all these data we come to the result that zamzam water affect uterine fibrochondro-sarcoma cell line through affection of gene downregulation and following this the function of gene which was affected.

## CONCLUSION

Zamzam water is a miracle. It affects interleukin matrix metalloproteinase tissue inhibition and insulin like growth factor binding protein 3, and nuclear factor of kappa, of primary cell line of uterine fibrochondrosarcoma.

## REFERENCES

1. **Naeem N, Alsanussi H, and Almohandis A (1983):** Multielemental and hydrochemical study of Holy zamzam water. Journal new England water works Association; 47: 158.
2. **El-Zaiat SY (2005):** Group Refractive index measurement by Frings of equal chromatic order. Opt, and lasers Technol 37: 181.
3. **El-Kashef H (1994):** Optical and electrical properties of materials. Rev Sci Inst 65: 2056.
4. **Ali Farid Mohamed Ali:** Oncolytic action of zamzam water on rat colon tumor 2008 (unpublished).
5. **A snapshot of colorectal cancer** [<http://prg.nci.nih.gov/snapshots/Colorectal-Snapshot.pdf>]
6. **Ah-Kim H, Zhang X, Isla s, Sofi JI, Glickberg Y, Malemud CJ, Moskowicz RW, Haqqi TM (2000):** tumour necrosis factor  $\alpha$  enhances the expression of hydroxyl lyase, cytoplasmic antioproteinase-2 and a dual specificity kinase TTK inhuman chondrocyte like cells. Cytokine 12 (2), 142-150.
7. **Amin AR (2000):** Gene mining, bioinformatics and functional genomics in human arthritis and inflammatory disease ex vivo. Drug Dev. Res 49, 22-29.

8. **Archer CW, Francis-West P, (2003):** The chondrocyte. *Int J Biochem Cell Biol* 35(4): 401-404.
9. **Booth C, Hargreaves DF, Hadfield JA, McGown AT, Potten CS (1999):** Isoflavones inhibit intestinal epithelial cell proliferation and induce apoptosis in vitro. *Br J Cancer* 1999, 80:1550-1557.
10. **Cawston T (1998):** Matrix metalloproteinases and TIMPs: Properties and implications for the rheumatic diseases. *Mol Med Today* 4(3): 130-137.
11. **Clancy BM, Johnson JD, Lambert, AJ, Rezvankhah, S, Wong, A, et al. (2003):** A gene expression profile for endochondral bone formation: Oligonucleotide microarrays establish novel connections between known genes and BMP-2 induced bone formation in mouse quadriceps. *Bone* 33(1), 46-63.
12. **Cohen JA, Weiner DB, More KF, Kokai Y, Williams WV, Maguire HC Jr, LiVolsi VA, Greene MI (1989):** Expression pattern of the neu (NGL) gene-encoded growth factor receptor protein (p185neu) in normal and transformed epithelial tissues of the digestive tract. *Oncogene* 1989, 4:81-88.
13. **De Potter CR (1994):** The neuroncogene: more than a prognostic indicator? *Hum Pathol* 25:1264-1268.
14. **Eviatar T, Kauffman H, Maroudas A (2003):** Synthesis of insulin-like growth factor binding protein in vitro in human articular cartilage cultures. *Arthritis Rheum* 48(20): 410-417.
15. **Googs R, Carter SD, Schulze-Tanzil G, Shakibaei M, Mobasheri A, (2003):** Apoptosis and the loss of chondrocyte survival signals contribute to articular cartilage degradation in osteoarthritis *Vet J* 166(2): 140-158.
16. **Hakkak R, Korourian S, Ronis MJ, Johnston JM, Badger TM (2001):** Dietary whey protein protects against azoxymethane-induced colon tumors in male rats. *Cancer Epidemiol Biomarkers Prev* 10:555-558.
17. **Kim BS, Seo JC (2003):** Microarray analysis of hypoxia-induced changes in gene expression in BV-2 microglial cells. *J Kor Acup Mox Soc* 20(4): 85-92.
18. **Chang Shikrin, Hee Jaelee, Seung-Jae Hong et al.:** Microarray analysis of gene expression in chondrosarcoma cells treated with bee venom *Toxicon* 2005; 45: 81-91.
19. **Lam J, Abu Amer, Y, Nesloon CA, Fremont, DH, Ross, FP, Teitelbaum SL (2002):** Tumour necrosis factor superfamily cytokines and the pathogenesis of inflammatory osteolysis. *Ann Rheum. Dis* 61: ii 82-ii83.
20. **Lee W, Kang s, Koh, H, (2003):** Microarray analysis of CD/ cytokine gene expression in human mast cell treated with bee venom. *J Kor Acup Mox Soc* 20(5): 50-62.
21. **Liacini A, Sylvester J, Li, WQ, Huang W, Dehnade F, Ahmad M, Zafarullah, M (2003):** Induction of matrix metallo proteinase 13 gene expression by TNF-alpha is mediated by MAP kinases, AP-1 and NF-kappa B transcription factors in reticular chondrocytes. *Exp. Cell Res* 288(1): 208-217.
22. **Moon C, Soria JC, Jang SJ, Lee J, Obaidul Hoque M, Sibony M, Trink B, Chang YS, Sidransky D, Mao L (2003):** Involvement of aquaporins in colorectal carcinogenesis. *Oncogene* 22:6699-6703.

23. **Morales TI (2002):** The insulin like growth factor binding proteins in uncultured human cartilage: increases in insulin-like growth factor binding protein 3 during osteoarthritis. *Arthritis Rheum* 46(9): 2358-2367.
24. **Thornton, S, Soders, D, Aronow, B, Witte, DP, Brunner, HI, Giannini, EH, Hirsch R, (2002):** DNA microarray analysis reveals novel gene expression profiles in collagen-induced arthritis *Clin. Immunol* 105(2): 155-168.
25. **Vincenti MP, Brinckerhoff CE (2001):** Early response genes induced in chondrocytes stimulated with the inflammatory cytokine interleukin-1 $\beta$ . *Arthritis Res.* 3: 381-388.
26. **Yanagihara K, Ito A, Toge T, Numoto M (1993):** Antiproliferative effects of isoflavones on human cancer cell lines established from the gastrointestinal tract. *Cancer Res* 1993, 53:5815-5821.
27. **Yang YH, Duodit S, Luu P, Lin DM, Peng V, Ngai J, Speed TP (2002):** Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systemic variation. *Nucleic Acids Res.* 30(4), e15.