



Bioreaction of the Cells against the Stress Burden

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INTRODUCTION

All the livings on the earth exposed to the undesirable surrounding after their birth as temperature, chemicals, less nutrition, radiation and others, but they had to live for the next generation. by overcoming these problems with their own hands, concretely they generate the special protein Heat shock protein (Stress Protein) to repair the stress-caused degenerated protein. to survive (1). In this text, the electron micrograph of HSP extracted *Mycobacteria bovis* BCG, was first shown, followed by immunological cross reaction with a variety types of tumor cells suggesting to be applicable as the tumor new markers. Further, the biofunction induction from the natural resource was introduced and discussed on our experiments of the black garlic and shell fish.

MATERIAL AND METHOD

- *Mycobacteria bovis* BCG HSP antibody was prepared by the Sasaki et al, and Zn deficient medium cultivation by Dr De Bruyn in The Brabant Pasteur Institute Belgium. These MoAbs were used sed for cross reactivity analysis with tumor cells by the fluorescein microscopic methods.
- Tumor c ells used for cross reactivity test; Meth A, Colon 26 in mouse and human bladder cancer cells established as strain in our laboratory.
- Baked Sea Shells powder for bioassay; Rinsed in water *Corbicula japonica* shell were baked at 150~750 2 hours by programmed temperature control machine (AT-E 58, ISUZU) Co. Ltd., Japan) and the crystal structure was analyzed by the X ray diffraction apparatus (XD-610, Shimazu, Japan).

RESULTS

Electron Microscopy View of *Mycobacteria bovis* BCG HSP 64 kDa Protein

As seen in Photo. 1, the BCG HSP 64kDa is the **donut shape** with a central hall to catch up the heat (stress)-caused denatured protein (left two in Phto1) (4). The denatured protein was carried to the HSP Center hole to repair to the normal. Similar phenomenon might occur in cancerous cells and bacteria, that is a reason of.

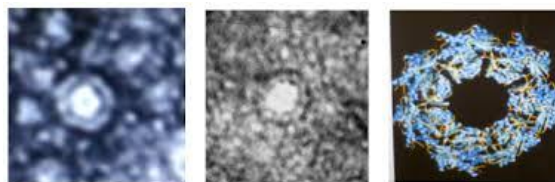


Photo 1: Electron Micrograph of BCG HSP 64 and the Computer designed HSP 64

BCG HSP 64 Ab. and Tumor Cell Immune Cross Reactivity

Tumor cells reactivity of *Mycobacteria bovis* BCG HSP monoclonal antibody with various type of tumor cells were analyzed by fluorescein microscopic methods, and shown in Photo. 2

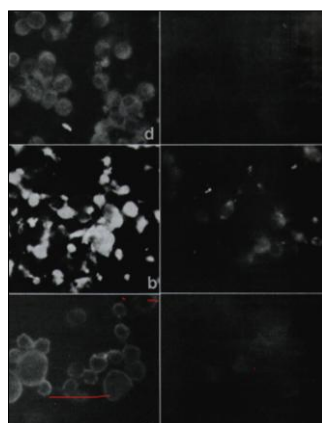


Photo 2: Indirect Immune Microscopy Analysis of Cross Reaction between BCG,64 and Mouse, Human tumour cells

From upper left lane, Meth A , Colon 26 in Balb C mouse.and Human Bladder cancer cells. reated with FITC-BCG Ab.andRight lane is cprntrol treated with norm serum instad of antibody (5)

Heat Stress Made Black Garlic

In 2005 the multi-function armed new vegetable the black garlic was created in south part Mie prefecture by heating of raw garlic at around 60-70 C. for a month. This black garlic is sweet with less irritating smell demonstrating the anti-tumor activity in mouse tumor mode, and enforced the anti-oxidant activity (Table 1).

Table 1: Enforced Anti-Oxidant Activity of the Black Garlic

Products	Black Garlic	White Garlic	RS. (50% to Control)
Japan	4.1 mg	115 mg	28 times to Cont.
China	7.3	39 mg	times to Cont.

*mg used to reduce 50% of 1.1-diphenyl 2-picrylhydrazyl

General garlic was heated at 60-70 °C for a month without any additives and treatment. Heating stress is inevitable and preferable for the raw garlic maturation to induce new biofunction. To make sweet spinach, the farmers expose spinach to snow until spring and give low temperature stress to induce the sweetness. They had already known this method and applied for marketing with brand name of 'Winter Made Sweet Spinach' at special high price.

Biofunction of the Heat-Treated Sea Shells Powder

Research historical ground is going back the Edo Period the pilgrim monk felt down with hunger. Villagers cared him. Recovered monk treated the disease people as thanks took medical care for villagers with the baked shell powder. Rinsed in water *Corbicula japonica* shell and dried were baked at 150-750 °C 2 hours and milled for powder by the mill machine. Powder sample provide for the X ray crystallography to analyze structure and obtained result below Table 2).

Table 2: Crystal Structure Change of Shell by Heat Treatment (X ray crystallography Analysis)

Temperature	Treated Hours	C. Structure
105 °C	2 hrs	CaCO ₃ Aragonite
250	-	CaCO ₃ Aragonite
500	-	CaCO ₃ Calcite
	-	Lime

Temperature dependent crystals structure change by Heat treatment. *Corbicula japonica* shell fish meat had been traditionally used as a folk medicine to improve the liver functional disorder. The local government requirement to scientifically confirm of the monks talks. Amazingly the heated shell powder at 500 °C (Calcite shell powder was very effective to improve the liver function defect LEC Rat Model demonstrating to lower the GOT, GPT and TBil value in serum. This animal result was confirmed in the volunteers as shown here

Effective Work of Calcite Shell Powder in Human Volunteer with Liver Mal Function (6)

We could successfully confirm the CEL rat result in the human volunteer too. Further we lately found the additional new bio-function of the anti-diabetes to lower the blood sugar. Likely by an appropriate heat controlling it is possible to pull out a new biofunction from the materials.

Table 7. Clients report based on medicinal check up

Client no.	Items and values	(Before & after)	Initial date to use and up to
1) N.T (F,81y)	γGTP	? 146	1/2016 8/2018
	AST	143 26	
	ALT	124 27	
2) A.H (M,64y)	γGTP	366 146	7/2014 6/2015
	AST	89 26	
	ALT	91 27	
3) S.K (M,66y)	γGTP	500 131	5/2012 1/2013
	AST	95 32	
	ALT	53 17	
4) Y.H (M,49y)	γGTP	37 25	7/2016 4/2017
	AST	58 19	
	ALT	93 21	
5) M.R(M,52y)	γGTP	168 72	6/2010 9/2010
	AST	40 29	
	ALT	44 25	
6) O.K (M,49y)	γGTP	67 43	6/2016 5/2018
	AST	50 24	
	ALT	85 31	

CONCLUSIVE CONSIDERATION

Living creatures on earth constantly facing to the stress burden and they produce the HSP to repair the stress caused denatured protein, or the black garlic). In the black garlic it generates the S-allyl-L-cysteine to enforce immunity, anti-oxidant activity. Further, the heat-treated shell demonstrated the liver function improvement effect and antidiabetic, and lipid lowering activity too. All these biological new activities were induced by just heating the materials without any additives. The heating power contains more feasibility in future to explore.

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