

COLON CANCER SPECIFIC COLON TISSUE PROTEINS

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SUMMARY

In this work the protein distribution was examined in the tumoral and normal parts of the colon tissue of colon cancer patients. The protein fractions were isolated either by percholoric acid precipitation (cases :1_8) or by ammonium sulphate precipitation (cases :9_10). These protein fractions were analyzed by SDS-PAGE. CEA levels were determined and CEA was detected both in the normal and cancerous parts. The protein fractions of the cases 9 and 10 were also analyzed with isocratic HPLC. Protein peak with Rts 11⁶⁰ and 12⁰⁰ were observed in the tumoral and samples, respectively.

KEY WORDS

Colon adenocarcinoma and antigens, colonic carcinoma, colon cancer, carcinoembryonic antigen, colorectal carcinoma

INTRODUCTION

With the progress in serological methods, tumor specific antigens (TSA) to each individual patient having the same tumor types eg- adenocarcinoma are being described. Recently TSA are being investigated with respect to their use in diagnosis or in monitoring the therapy in cancer cases. In this work we tried to isolate tissue antigens specific to each patients in the colon tissue material with adenocarcinoma. (1,2,3,4,5)

MATERIAL AND METHODS

The colon material was isolated through surgery from the patients of surgery department of Istanbul University Cerrahpaşa Medical School Hospital. The colon material was transferred to our laboratory immediately in physiological serum. The specific proteins as TSA are isolated according to the method of M.S.Kleinman, S.Von.Kleist and M.D. Turner (1,6,7)

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THE ISOLATION OF PROTEINS FRACTIONS BY PERCHLORIC ACID PRECIPITATION FROM THE COLON MATERIAL

The colon material was separated into normal and pathological parts. Each colon section was homogenized for 1 h in physiological serum. Equal volume of Perchloric acid (1.2 mol/l) was added and stirred for 10 min. at room temperature. Homogenized again for 1/2 h. Stirred again for 20 min and centrifuged at 4C for 15 min at 10.000 rpm. (Beckman -J 21 B, Rotor No JA 20) The supernatant was saved as S1. Original volume of Perchloric acid was added again to the precipitate stirred for 30 min. at room T and centrifuged for 30 min at 4C for 15 min. at 10.000rpm. Supernatant was named as S2. S1 and S2 were mixed together and dialyzed against distilled H₂O at 4C. After dialysis it was centrifuged at 4C for 30 min. at 10.000 rpm. Supernatant was concentrated with lyophilization to 1/4 of its volume (1,6,7)

THE ISOLATION OF PROTEIN FRACTIONS BY AMMONIUM SULFATE PRECIPITATION FROM THE COLON MATERIAL

The normal and tumor (adenocarcinoma) parts were separated from the colon material. Equal weight of each part was homogenized for 1 h. at 4C in distilled water. Equal volume of Perchloric acid (10%) was added and centrifuged immediately at 10.000rpm. for min 30 at 4C. The supernatant was neutralized with 6N NaOH and saturated with (NH₄)₂SO₄ centrifuged and precipitate was suspended in minimum volume of 0.15 mol/l. Tris-HCl buffer (pH 8.2) and dialyzed for 34 hours at 4C against same buffer and lyophilized for concentration to its 1/4 volume (8)

PROTEIN DETERMINATION : Protein assay was performed according to Lowry Method, Protein fractions were analyzed with SDS-6H PAGE and with isocratic HPLC. CEA was determined immunologically (9,10,11,12)

RESULT AND DISCUSSION

Protein concentrations in 6 of the 10 cases the total protein was increased in the cancerous part and in 3 cases (cases no:4,6,7) the protein concentration was high in g tissue basis in cancer part (Table I, Table II and Table III).

SDS_PAGE: The protein fractions were studied in the cases of 1 to 8 with perchloric acid precipitation and in cases 9 and 10 the proteins were isolated by ammonium sulphate precipitation.

HPLC: Waters HPLC with 481 detector, variable wavelength, 30 model data module, 510 modal pump was used with Protein PAK-300 W column. The flow rate was 0.9 ml AUFS-0.02, the rate of the paper was 0.5 cm. and the wavelength was 280nm.

The SDS-6H PAGE of result normal and tumorous parts of colon material and of standarts are seen in Table III. The molecular weight of proteins in tumor part differing from the normal part varied from 5600 Da to 96000 dA. Being different in each colon material.

The HPLC graphics result of normal and tumorous colon material are seen in Fig. I with their retention times. The retention times varied in normals from 12⁰⁰ to 11⁶⁰ and in tumorous parts the retention times were found between corresponding to different proteins with different molecular weights RT values.

CEA: The CEA values of normal and tumor colon material compared to normal tissue values the tumor tissue didn't show any significant change into two cases determined (Table IV)

TABLE I THE CHARACTERISTICS OF THE COLON MATERIAL

CASE NO	SEX	TISSUE MATERIAL		PROTEIN CONCENTRATION	
		NORMAL	TUMOROUS (gr)	NORMAL (µg/ml)	TUMOROUS
1	M	7.8	6.20	300	185
2*	M	---	5.10	---	225
3	M	a2:5.3	a1:8.2	a1:370	2:305
4	M	4.75	4.30	210	250
5	M	6.2	6.25	372	320
6	---	6.0	5.50	120	200
7	M	6.5	8.00	125	225
8	---	6.2	6.00	175	170
9	F	9.0	9.00	190	175
10	F	11.0	11.0	4.45	3.50

*Experiments were performed as duplicate in whole colon tissue, were shown necrosis on it

**Protein fractions were obtained by perchloric acid precipitation from cases, number 1-8.
Protein fractions were obtained by ammonium sulphate (100%) precipitation from cases, number 9-10

TABLE II SDS-PAGE MOLECULAR WEIGHT RESULTS OF PROTEIN BANT

CASE NO	Tumorous colon (MW)	Normal colon (MW)
1	56000	24000
2	26000	---
3	55000	24000
4	1 bant 96000 2 bant 66000	68000
5	64000	51000
6	No protein bant was detected	
7	No protein bant was detected	
8	26000	---
9	No protein bant was detected	
10	54000	72000

TABLE III The amount of protein obtained from lyophilisation in the total volume and (µg) tissue.

CASE NO	NORMAL	TUMOROUS
1	0.076	0.029
2*	---	0.11
3	a1:0.12	a2:0.13
4	0.09	0.10
5	0.14	0.13
6	0.05	0.07
7	0.05	0.11
8	0.07	0.06
ort SD	*0.085 ± 0.014	0.087 ± 0.013

* No significant differences

TABLE IV: CEA

CASE NO	CEA (ng/ml)	PROTEIN (µg/ml)	*HPLC (ml)	**CEA (ng/ml)	CEA
normal (9)	18	190	1.0	15	15.0
tumorous (9)	20	175	2.5	50	12.5
normal (10)	210	4.45	2.0	2100	4200.0
tumorous (10)	125	3.50	1.5	447	670.5
tumorous	---	---	1.0	259	259.0

*Collected volume, **In collected volume

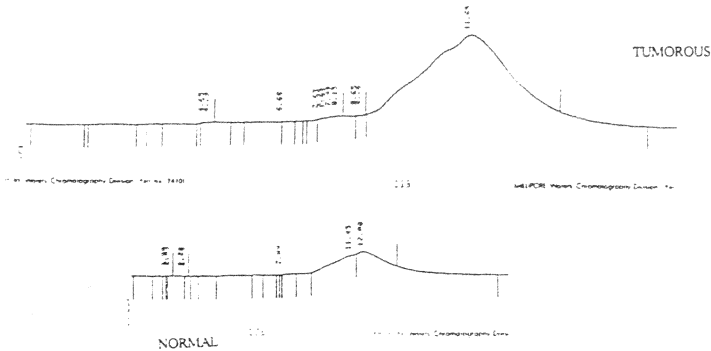


Fig 1

CONCLUSION

Protein isolated according to M.S.Kleinman. et. al., method showed differences in the tumor tissue of adenocarcinoma patients. In the 10 patients analyzed each patient had a different protein of different molecular weight. The molecular weights of these proteins varied from 5600 Da to 96000 Da. These results might signify that specific proteins to each colon cancer patients although they are all diagnosed as adenocarcinoma due exist and they can be classified as tumor specific antigens. Following these specific proteins in the serum of each patient could be very valuable in detection the progression and remission of the patients. Increases in the experimental cases and to work with high yields of protein tissue culture experiments and preparation of monoclonal antibodies to patients specific proteins is our future aims (13,14,15).

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