Journal of
Carcinogenesis & Mutagenesis

Gianfranceschi and Reichelt, J Carcinog Mutagen 2015, 6:3
DOI: 10.4172/2157-2518.1000226

Research Article Open Access

Low MW Peptides and Carcinogenesis

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Received date: March 30, 2015; **Accepted date:** May 21, 2015; **Published date:** May 27, 2015

Abstract

Problem: Low MW peptides many of them N-substituted, have growth inhibitory effects. Are the peptide levels different in malignant cells from normal cells?

Method: Deprimerones dissociated from DNA at pH 9.5 was measured in normal and malignant cells. The level of Low MW compounds was also compared to large MW compounds [ratios] after gel filtration.

Result: Chalones and some other low MW compounds were not as in normal tissues and cells found in these. However, these peptides could be found in the incubation fluid or ascites. DNA bound peptides were decreased in malignant cells.

Keywords: Peptide; Transcription; Inhibition; Differentiation; Carcinogenesis

Introduction

Chalones [1] are endogenous growth inhibiting factors with reversible and relative tissue specific effects. Purification however, was initially not successful, and we now know that this was due to low MW peptides binding to different protein molecules and other macromolecules depending on concentration of salts, pH etc . Peptides easily bind to larger molecules and each other [2]. Deprimerones also peptides, bind to DNA [3]. Chalones and deprimerones are all N-substituted (Table 1). They often have bell shaped [hormetic] dose responses and demand extensive testing over a large concentration range. Optimal effects are often in the nano-to pico-molar range. The Chalones and deprimerones are externalized or lost from the cells in malignant states [4-6] and can be purified from ascites [6].

Properties of low MW mitosis inhibitory peptides

A: They are apparently phosphorylated by protein kinase CKII and can translocate to the nucleus where they bind to DNA [11-13] but not by a covalent bond [14].

B: These peptides cause differentiation when inhibiting mitosis [15-16], which is the opposite of the malignant process.

C: The peptides apparently act by controlling transcription [3,13,17-19].

D: The chalones show bell shaped dose responses with optimal effect from 10-9 to 10-14 M [1]. This phenomenon is known as hormesis.

E: Cyclic AMP is involved since propranolol modifies the effects of epidermal penta-peptide [20]. The colonic tri-peptide also decreases non-tumorigenic colon cells levels of cyclic AMP [21].

F: The epidermal peptide and the hemo- regulating peptide change RNA profiles in target cells [22]. Microarray of oncogenes ± specific peptide to cultured T cells or colon carcinoma cells [HT29] studied with real time PCR [23-25] points to similar mechanisms. The epidermal pentapeptide inhibits the oncogene c-Fos, ki-ras and Neu m-RNA formation in TC3H10 cells [23]. The Colon tri- peptide caused a considerable increase in Fos antigen [24].

G: Phosphorylation also make the peptides more resistant to peptidase break down [12].

H: The binding to DNA seems to be divalent cation [Mg^{2+}, Fe^{2+}, Cu^{2+}] dependent [18].

I: Different growth inhibitors impede metastases and growth of subcutaneously injected tumors [26-29] and most in the slowly growing clones. The Colon tri-peptide also inhibits cholic acid induced hyperplasia and hypertrophy [30] as well as Trimethylhydrazine induced hyperplasia [31].

J: The peptides "leak out" or are transported out to the medium from malignant cells or tissues compared to normal cells and tissues [4,5]. For instance the mammary carcinoma cell inhibitor could be found in the ascites fluid [4-6].

K: Combining the Colon derived tri-peptide with Vitamin A [also a differentiation inducing factor] enhances the effect of the peptide against HT29 considerably [26]. The cancer cells were injected in athymic mice [26] with inhibition of more than 90%.

Epidermal cells	PyroE-E-D-S-GOH and PyroE-GOH	1
Colon Endothelial cells	PyroE-H-GOH	1
Hepatocytes	PyroE-Q-S-G-DNH2, PyroE-Q-S-G-DOH, Pyro-E-E-S-G-DNH2, PyroE-E-S-G-DOH.	1
Melanocytes	PyroE-F-GNH2	1

T-Lymfocytes	AcE-S-GNH2	1
Neuroblast cells	AcD-Q-Y-GNH2	
Hemoregulatory peptides	Pyro-E-E-D-C-KOH AcS-D-K-POH].	7 8
Thymus factors	pyroE-A-E-S-N; PyroE-A-G-G-S-E-D PyroE-A-G-E-E-S-N	9
Seminal plasma	PyroE-A-E-S-A PyroE-V-A-D-S-D-Q-N	1 0
PyroE=pyroglutamic acid.		

Table 1: Isolated low MW growth inhibitors of chalonic nature.

Methods

Deprimerones were isolated from different tissues as described [3] and the peptide released from DNA by alkaline extraction in a bicarbonate buffer at pH 9.5. Peptide levels are expressed as µg peptides/10 mg DNA.

The following normal tissues were studied: rat Liver, mouse Liver, mouse Thymus and Fibroblast L-929 cells. The malignant cells were Novikoff hepatoma cells, mouse fibrosarcoma, mouse lymphosarcoma and fibrosarcoma LP-59 cells.

Chalones were obtained by immersing tissues or cells in ice-cold water [4], and homogenized in ice. Acetic acid was added to a final concentration of 0.5M which also inhibits many peptidases. Proteins were separate from low MW compounds by gel filtration on Sephadex G-25 columns in 0.5 M acetic acid.

Aliquots of 0.4 ml each 4 ml fraction were hydrolyzed in 2 M KOH for 2 hours in a boiling water bath, neutralized with 2 MHCl and ninhydrin color developed as described by Rosen [32] [also tryptophan survives this hydrolysis]. With this method each amino acid has the same molecular absorption coefficient. The following cells and tissues were studied: Normal tissues: Epidermis [Mouse], Epidermis [human], Epidermis [Pig], Colon [Mice], Colon [Human], Liver [Rat], Liver [Mouse], Cortex [Pig], Kidney [Dog], Spleen [Pig] and granulocytes [Human]. Malignant tissues were from epidermal sarcoma [Human], colon carcinoma [Mouse], colon carcinoma [Human], hepatoma [MH1C1, Rat], neuroblastoma [Human], melanoma [Human], Myelogenic leukemia [Human] [3,4].

The ratio of low MW ninhydrin colorable compounds to the post hydrolysis amino acid content of the protein peak from G-25 was calculated for each experiment.

Results

A clear cut decrease in DNA binding peptides as well as all post hydrolyses ninhydrin colorable low MW compounds were found in cancer cells and tissues (Tables 2 and 3). The increase growth rate could be stopped by reintroducing the total peptide level to the normal one .

Similarly the level of amino acids and peptides [low MW fraction after G-25 filtration] was significantly reduced in malignant cells and tissues as seen in Table 3 [4,5].

The inhibitory peptides could be isolated from incubation fluid or ascites, but very low yields or not at all from the malignant cells and tissues [4,5].

Cell type	Peptide level	± SEM	N	compared	P
Novikoff hepatoma	95	18	3		
Rat liver	179	24	3		
Mouse liver	185	10	3		
All normal liver cells	177.5	9.8	6	1 and 4	0.0078
All normal cells	187.4	7.9	12		
All malignant cells	116.8	7.0	12	5 and 6	0.0001

Table 2: Level of active peptides released at pH 9.5 from DNA.

Peptide level in µG peptide/10 mg DNA. The remaining peptide fraction when added to a concentration of 10 µg peptide fraction/5µg DNA inhibits RNA polymerase by approximately 92%.

Cell type	ratio	SD	N	compared	p
Malignant tissues	4.18	0-9	20		
Normal tissues	0.83	0-39	19	1 and 2:	0.0001
Malignant cells	4.48	1.43	9		
Normal cells	0.17	0.48	5	3 and 4:	0.001
All malignant cells/tissues	4.49	0.84	40		
All normal cells/tissues	0.980	1.39	41	5and 6:	0.0001

Table 3: Change in Protein/Low MW ratios in normal and malignant cells and tissues.

The table shows the ratio of the protein peak from G 25 divided by the low MW compounds, both measured as post hydrolysis released ninhydrin colored material. The low MW compounds are lost from the transformed and malignant cells investigated by gel-filtration.

Discussion

Both the deprimerones isolated by the Italian group and the chalones are decreased in malignant cells and tissues compared to normal controls. When the brakes to growth and/or mitosis are removed faster cell growth is to be expected. Cells that have a high mitotic rate more easily turn malignant [33].

If decreased levels of inhibitor are critical then peptidases and proteases that break down these peptides [34] ought to be involved in carcinogenesis. Increased break down of peptides seem to increase tumor growth. Thus increase in membrane associated cathepsin L increases metastasis of melanomas [35]; Increase in cysteine endopeptidase [36], and Cathepsin D in breast cancer [37] indicate such a possibility. Increasing peptide levels by inhibiting break down [38] seem to retard tumor growth. Peptidases and proteases may have

prognostic value [39,40]. Furthermore the more malignant some tumors are the stronger is the out transport from the cell [41,42]

Based on our findings we propose that decrease in peptides and externalization of the same, may be a final common path in carcinogenesis. Is it possible that membrane changes resulting in decreased level of inhibitory peptides and amino acids is the primary lesion in carcinogenesis?? This would fit both the transport or diffusion out of cells and the peptidase data, where increase in peptidase activity promotes malignancy while decrease inhibits tumor growth.

Conclusion

Low MW peptides that are mitosis inhibitors and differentiation factors are decreased in transformed and malignant tissues and cells. The chalones can be recovered from the growth medium or from ascites fluid. Differentiation decreases with increased growth and mitosis rates. Increased growth rate ought to increase possible mutation rates and possibly carcinogenesis?

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