Low MW Peptides and Carcinogenesis

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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 deprimorones are all N-substituted (Table 1). They often have bell shaped [hornet] 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 deprimorones 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 propanolol 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 [Mg²⁺, Fe²⁺, Cu²⁺] 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
1

Melanocytes
PyroE-F-GNH2
1
tryptophan survives this hydrolysis. With this method each amino acid could be stopped by reintroducing the total peptide level to the normal water [4], and homogenized in ice. Acetic acid was added to a final concentration of 0.5M which also inhibits many peptidases. Proteins were separated from low MW compounds by gel filtration on Sephadex G-25 columns in 0.5 M acetic acid.

Liver [Rat], Liver [Mouse], Cortex [Pig], Kidney [Dog], Spleen [Pig] peptides/10 mg DNA.

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.

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].

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

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Peptide level ± SEM N</th>
<th>compared</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novikoff hepatoma</td>
<td>95 ± 7.0 13 1.39</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Rat liver</td>
<td>179 ± 7.0 24 0.84</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Mouse liver</td>
<td>185 ± 7.0 10 1.43</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>All normal liver cells</td>
<td>177.5 ± 7.0 9.8 6 3 and 4:</td>
<td>0.00078</td>
<td></td>
</tr>
<tr>
<td>All normal cells</td>
<td>187.4 ± 7.0 7.9 1 2</td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td>All malignant cells</td>
<td>116.8 ± 7.0 7.0 1 5 and 6:</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

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%.

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

The table shows the ratio of the protein peak from G 25 divided by the low MW compounds, both measured as post hydrolysis 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...
and differentiation factors are decreased in transformed and malignant tissues and cells. 

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?

References

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