



Selective targeting of signet ring cell adenocarcinomas

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ABSTRACT

Many epithelial tumors, especially signet-ring cell adenocarcinomas, produce huge amounts of mucin glycoproteins that fill cytoplasm and push nucleus to the periphery, giving a signet ring like structure to the cell. Mucin proteins are very rich of l-threonine which is essential in humans. L-threonine content can reach up to 35% of total amino acid composition of some mucin proteins. Therefore l-threonine can be the Achilles heel of signet ring cell adenocarcinomas which are one of the most malignant and aggressive cancers. A modified bioisostere of l-threonine, 4-fluoro l-threonine (its fluorine can be radioactive or not), can be used to selectively kill signet ring cancer cells without harming normal cells or for diagnostic purposes.

Background

Some types of cancer cells produce large amounts of mucin protein. These cells look like signet-rings under microscope due to the excess mucin protein accumulated in their cytoplasm. Mucin pushes the nucleus to the periphery of the cell. The adenocarcinomas represented with these cells are called as signet-ring cell carcinomas (SRCC) [1,2].

Although SRCC tumors are found mainly in the stomach, they can also be found in breast, colon, bladder, prostate and any other primary organs [3]. Due to their aggressive character and late stage diagnosis, many difficulties are encountered in their treatment [4]. SRCC incidence increases each year and makes up about 35–45% of gastric adenocarcinomas in Asia, United States of America and Europe [5,6]. The incidence of the disease was increased ten folds in the years between 1970 and 2000 [7,8].

Mucin proteins are characterized by amino acid repeats rich of l-serine, l-proline, and l-threonine [9]. Among these amino acids, only l-threonine is essential and must be provided with diet. Recommended dietary daily allowance for l-threonine in humans is 87, 37, 28, and 7 mg/kg/day for 3–4 month, 2 year, 10–12 year olds, and adults respectively [10].

Mucin proteins are categorized into two groups as transmembrane and secreted (gel-forming) [11]. Mucin proteins MUC1, MUC3A, MUC3B, MUC4, MUC12, and MUC17 are transmembrane; MUC6, MUC2, MUC5AC and MUC5B are secreted [12–14]. Secreted mucins make the sticky mucous layer that covers the epithelial surfaces of human body as a protecting barrier [15,16].

The total count of l-threonine in mucin proteins is up to 35%, which is higher than any other amino acid. The list of the 15 highest threonine containing mucin proteins were listed in Table 1.

The hypothesis

Mucin-rich cancer cells can be targeted using modified l-threonine that can participate in protein synthesis. Radioactive l-threonine produced by substitution of its carbon, oxygen and hydrogen atoms with their corresponding radioisotopes cannot yield sufficient radioactivity dosage for their use in systemic radiotherapy or scintigraphy. Because, they have a very low energy. But substitution of one of its hydrogens with radioactive fluorine can solve this problem.

Fluorine is a bioisostere of hydrogen [17], changing one or some of the hydrogens in threonine with fluorine is not expected to affect threonine's use in protein synthesis.

Fluorine containing organic compounds are very rare in nature. The first example discovered is 4-fluoro-L-threonine. It is synthesized by a gram-positive bacterium, *Streptomyces cattleya* [18], and commercially available.

The fact that signet ring cells contain too much mucin proteins and mucin proteins contain up to 35% l-threonine, the targeting of these cells by 4-fluoro L-threonine can assure its toxic accumulation and selective killing before reaching toxic concentrations in normal cells.

Since 4-Fluoro-l-threonine is produced by a bacterium, it can be expected to be incorporated into protein by protein synthesis machinery. Non-radioactive 4-Fluoro-l-threonine is toxic on some bacteria, therefore it can show the same effect by accumulating on mucin ring cells also. In rats, the LD50 dose is 320 mg/kg in IV administration [19].

Evaluation of the hypothesis

For in vitro testing of this proposal, mucin-producing signet-ring cell cancer cell lines (i.e. NCI-H676B, HCC2998) [20,21] can be used to

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Table 1
Human mucin proteins and their threonine contents (Courtesy of UniProt team).

Mucin protein	Protein ID	Amino acids	Threonines	Threonines/total amino acids (%)	
1	MUC2	Q02817	5179	1810	34.95
2	MUC22	E2RYF6	1773	595	33.56
3	MUC3A	Q02505	2541	692	27.23
4	MUC17	Q685J3	4493	1081	24.06
5	MUC12	Q9UKN1	5478	1201	21.92
6	MUC3B	Q9H195	1237	260	21.02
7	MUC5B	Q9HC84	5762	1209	20.98
8	MUC5A	P98088	5654	1158	20.48
9	MUC19	Q7Z5P9	6254	1235	19.75
10	MUC21	Q5SSG8	566	109	19.26
11	MUC6	Q6W4X9	2439	413	16.93
12	MUC7	Q8TAX7	377	62	16.45
13	MUC16	Q8WXI7	22,152	3533	15.95
14	MUC1	P15941	1255	171	13.63
15	MUC4	Q99102	2169	285	13.14

observe if 4-fluoro L-threonine can enter protein synthesis and kill cells under culturing conditions in defined medium.

As an *in vivo* model, *Caenorhabditis elegans* (*C. elegans*) cultivated in defined *C. elegans* maintenance medium (CEMM) [22,23] can be suitable. *C. elegans* also contains a mucin ortholog protein called OSM-8 that is expressed in hypodermis [24]. OSM-8 contains a total of 331 amino acids and 29 (8.76%) of them are l-threonine [25]. The observation of 4-fluoro L-threonine's effect especially on hypodermis can provide important insight.

Consequences of the hypothesis

There are currently no selective treatment options for cancers with signet ring cells. In case of success of this proposed strategy, analogous to the success of radioactive iodine treatment in some thyroid cancers [26], it might open a new era in the treatment of cancers with mucin containing cells.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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