

Anticancer Effect in HL-60 Human Leukemia Cells and Other Health-Beneficial Functions of Cheese

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ABSTRACT

With regard to the aim of cancer prevention and/or treatment, a considerable number of basic studies have indicated that dairy and other plant-originated natural food products may possess anticancer activity. On the growth of human leukemia cells, for example, enzymatically digested skim milk or fermented milk cultured with various bacteria can exhibit differential suppressive activities. Our research team has previously revealed that highly ripened cheese was capable of demonstrating strong growth inhibition and induction of apoptotic DNA damage on HL-60 human promyelocytic leukemia cells. In this short review, the available information concerning potent anticancer effects of cheese was summarized. From the stand point of Food Science, functional implications for cancer prevention as well as multifaceted function of cheese are discussed.

Keywords: HL-60; Leukemia; Antiproliferation; Anticancer; Cheese; Food Function

1. Introduction

A concept of food function or functional foods has been raised in Japan [1]. This is one of the emerging fields in Food Science. For individual's health promotion and/or disease prevention, a word "food function" can be often used with the meaning of pharmacological effect of food-stuffs as well as their ingredients. Many studies have been energetically performed to seek the medicinal effects of various foods through the world. In view of cancer prevention/treatment, the anticancer effect of functional foods has not been approved in Japan because of the difference among medication and diet. However, numerous basic studies have demonstrated that a part of dairy and other plant-originated natural food products may possess anticancer activity [2-4]. On the cell growth of human leukemia cells, enzymatically digested skim milk of fermented milk cultured with various lactic acid bacteria and yeast can exhibit differential suppressive activities [5,6]. By serving as an apoptotic inducer on tumor development, compounds found in cow milk, e.g., lactoferrin and lactoferricin, can suppress the growth of cancer cells *in vitro* and *in vivo* [7-10]. In view of the cancer prevention, our research group has recently embarked on examining the antiproliferative effect of cheese in HL-60 human promyelocytic leukemia cells as

a model. The HL-60 cell line established in 1977 from a patient with acute myeloid leukemia [11], was chosen because of a valid and useful model for discovery of cancer chemopreventive or chemotherapeutic agents from natural products [6,7,12-14].

2. Anticancer Effect of Cheese

A previous study demonstrated differential antiproliferative activity of 12 different cow milk cheeses in HL-60 cells, which was used as a cancer model [15]. Similar findings have subsequently been made with 11 goat milk cheese in HL-60 cells [16]. An important issue raised is the mechanism underwent the antiproliferative activity. In our previous report, highly ripened cow milk cheese demonstrated higher antiproliferative activity, induction of apoptotic DNA fragmentation, and increase of nuclear morphological changes in HL-60 cells [15]. Based on the quantification of nitrogen contents in different cheese samples, a positive correlation was displayed between the ripeness of various cheeses and their antiproliferative activity found in HL-60 cells. In addition, four varieties of blue cheese ripened for 0, 1, 2, or 3 months demonstrated that the blue cheese ripened for a long term was capable of causing the strong suppression of the cell growth and induction of apoptotic DNA damage in HL-

60 cells. An incubation of cells in the presence of Pouligny Saint-Pierre goat cheese extract resulted in induction of cellular morphological change and apoptotic DNA fragmentation as well as expression of active form of caspase-3 protein, in a dose dependent manner [16].

A positive correlation between the lipolytic ripeness of various cheeses and their antiproliferative activity tested in HL-60 cells has been appeared [16]. Therefore, it is notable that intensity of the suppressive effects of individual cheeses found may vary with the length of ripening in each case. In view of immune regulatory function, our research team has demonstrated stimulating effect of cheese in nitric oxide generation by macrophages [17]. Whether cheese is capable of showing interferon- γ -like effect in immune stimulation, thereby contributing to play a role in tumoricidal effect is another interesting question. Nevertheless, further investigation should be given to whether the antiproliferative effect of cheese found is specific not to normal cells but to cancer cells.

3. Cell Specific Cytotoxicity

To investigate the cytotoxic effect of Pouligny Saint-Pierre goat cheese extract, an experiment was challenged using differentiated HL-60 cells and primary mouse splenocytes [16]. Because cancer cells are usually less differentiated than normal cells, cancer cells underwent differentiation may be used as a model closer to normal cells. It is also to note that HL-60 cells can be used in study for myeloid differentiation upon several inducer compounds. In our study, Pouligny Saint-Pierre cheese extract showed lower cytotoxic effect in differentiated HL-60 cells than undifferentiated HL-60 cells at the varying concentrations tested [16]. In the experiment using normal mouse splenocytes, no drastic decrease of the viable cell number was observed.

Although the results are still controversial, an epidemiological study has demonstrated that a high consumption of fermented dairy products, such as cheese and yogurt, is statistically associated with lower risk of cancer promotion [18]. It is interesting to note that oral administration of cheese or its derivative proteins, such as iron-bound lactoferrin and Ca-casein phosphopeptide, can suppress cell growth in Meth A fibro sarcoma-transplanted tumor model *in vivo* [19,20]. In addition, Cys-Cys and γ -glutamylcyst(e) in dipeptides originating from milk are thought to be efficient substrates for glutathione function, including detoxification of carcinogens, whereas lactoferrin and selenoproteins in milk can inhibit tumorigenesis [4].

Our recent evidences may indicate a potential role of cheese in suppressive effects on HL-60 cancer cell growth, and ripeness can be considered as a key factor. From a practical standpoint, whether active molecules, peptides,

free fatty acids, or others, taken from ripened cheeses may present at enough concentration in the human body to exert cancer-preventive function will be an interesting issue for further investigation.

4. Multifaceted Function of Cheese

Recent studies have raised an idea that certain compounds, especially peptides, found in fermented milk products may exhibit various physiological functions and also preventive effects against cardiovascular diseases [21,22]. A bovine casein-derived peptide generated during ripening of Edam cheese has been found to demonstrate inhibition activity in the intestinal absorption of γ -lactoglobulin [23], whereas other casein-derived peptides generated in fermented milk products have been shown to serve as antioxidative [24] and antihypertensive agents [25], respectively. A previous report also demonstrates a significant correlation between antioxidant activity of various cow cheese extracts and their peptide contents [26]. Our previous study has further implicated that some goat milk cheeses may possess antioxidant properties against reactive oxygen species, hydrogen peroxide and superoxide anion radical, wherein the amounts of constituents, phenolics, peptides, free fatty acids, and triglycerides in tested cheeses may potentially contribute to the intensity of their antioxidant activities [27].

In relation to bacterial lipolysis in cheese, free fatty acids have been demonstrated to be one of the key groups of compounds in directly regulating apoptotic cell death as well as lipid-metabolism-mediating cell death *in vitro* [14,28]. Several studies have demonstrated the presence of relatively minor but physiologically highly active free fatty acids such as conjugated linoleic acid, branched chain fatty acids, and short-chain fatty acids in cheeses, particularly with regard to their anticancer effects [2,29-32]. Studies have also implicated short-chain fatty acids generated from prebiotics in reducing the risk of developing gastrointestinal disorders, cardiovascular disease, cancer and inflammation [33,34]. Whether the active molecule(s) present in cheese exert(s) a cancer-preventive effect will be an interesting question for further investigation. The information obtained in this area will shed new light on the relevance of cheese as animal-originated fermented foodstuffs for prevention of cancer and other pathophysiological state.

5. Conclusion

In this short review, a potential role of highly ripened cheese in the prevention of leukemia cell proliferation *in vitro* has been described. Nevertheless, it will be an important area to examine what chemicals may affect the proliferation and the induction of apoptotic DNA damage as well as nuclear morphological changes on apoptotic

signal pathways occurred in HL-60 cells. Although it remains to be further examined, ripening of cheese may indeed serve to produce more of the biologically active compounds than are found in fresh cheese. Another area that needs to be explored further is what kinds of functional molecules have been generated during ripening in individual cheese. The information obtained may indicate a potential role of cheese in suppressive effects on cancer cell growth, and cheese ripeness can be considered as a key factor. More works are warranted to fully elucidate the health-beneficial contribution of cheese in the prevention of leukemia and possibly of other types of cancers.

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