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Rapid and synchronized dormancy-broken Kyoho grape seed endosperm without extraction or concentration shows significant effect on both anti-cancer and pro-immunity

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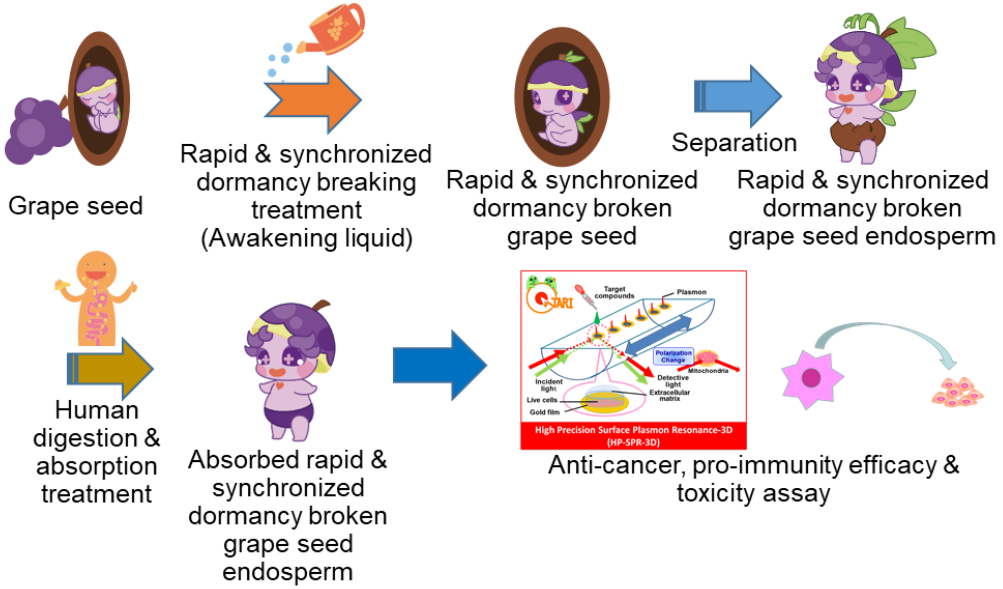
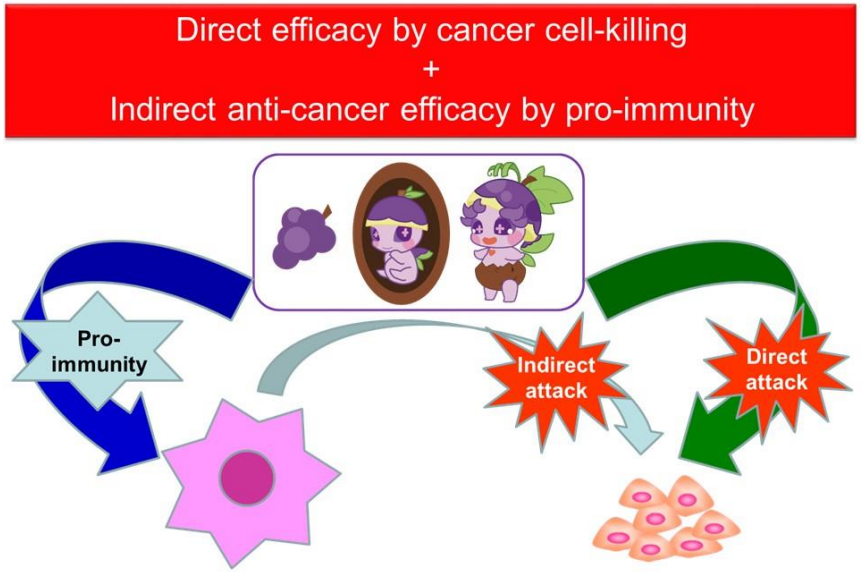
Graphical Abstract





Abstract: Anti-cancer effect has been reported in oral administration of grape seed extract (GSE) although the clinical trial failed because of its toxicity. Grape seeds should be toxic to prevent them to be eaten by animals. However, once they wake up, they change the ingredients into a form which dissolves easily in water and break toxicity substances inhibiting germination. In order for seeds to sprout and grow, a lot of nutrients as well as life itself reside. The objective of this study is to determine the in vivo-like efficacy and toxicity on anti-cancer and pro-immunity of rapid and synchronized dormancy-broken grape seed endosperm (RSDB-GSE) by our proprietary Grandir recipe. It was performed in case of oral administration using digested and absorbed RSDB-GSE. Direct killing efficacy against all cancer cells was observed in a dose-dependent manner at 2.5 mg/ml or more, and it was the maximum at 5.0 mg/ml for pancreas and liver and at 7.5 mg/ml for breast. The cell viability was less than 20%. At concentrations higher than these, it showed toxicity which was thought to be cell functional shutdown by suspension of mitochondrial polarization. No effect was observed for normal cells. The RSDB-GSE showed general direct cancer killing efficacy for organs. T lymphocytes cytotoxic activity by NK cells was observed in a dose-dependent manner at 3.75 mg/ml or more, and it was kept at least until 5.0 mg/ml. From this, pro-immunity was shown by RSDB-GSE. Consequently, it is expected to express synergistic anti-cancer effect, even orally administered.

Keywords: cancer; immunity; food; grape seed endosperm; dormancy-breaking

Introduction

Rapid and synchronized dormancy broken (awakened) Kyoho grape seed endosperm (RSDB-GSE) was prepared using a proprietary Grandir recipe™. After the seeds contents (ingredients) are converted by themselves, only the endosperm is separated. This process increases both anti-cancer and pro-immunity efficacies and decreases toxicity of the endosperm carried out at the Global Innovation Center, Kyushu University, Japan.

Background

Cancer disease causes the second of all deaths globally [1]. Grapes have been grown on the Caspian Sea coast and in the Caucasus since 3,000 BC and have a long history of eating habit [2]. Grape seed extract (GSE) is attracting attention because wine polyphenols are derived from fermented grape seeds [3]. GSE, a polyphenol extracted from grape seeds, has been reported to have anti-cancer effects and to reduce side effects of anti-cancer drugs and radiation therapy [4]. Unfortunately, a GSE clinical trial failed because of its toxicity [5, 6]. The main active substance, proanthocyanidin is a class of polyphenols, mainly contained in grape seed coats. Polyphenols are known as often causing toxicity [6].
Grape seeds are babies of grapes. They fall to the ground in autumn and pass through winter, and in spring, they wake up and sprout. To keep them alive for a long term, the ingredients of grape seeds are hard to dissolve in water so that they do not flow out even if it rains [7]. Furthermore, they should be toxic or bad taste threatening life to prevent them to be eaten by animals, whereas the seed coat cannot be digested. It is the same in humans, and those that are difficult to dissolve in water need to be changed into substances that dissolve in water in the body, which is inefficient and can cause toxicity either [8].

The need for assured efficacy with minimized toxicity as food

Grape seeds are in a dormant state in which growth activity is temporarily suspended until conditions are favorable for germination, with minimal metabolism and energy consumption [9]. However, once they wake up, they change the ingredients into a form which dissolves easily in water and break toxicity substances to inhibit germination [7]. In order for seeds to sprout and grow, a lot of nutrients as well as life itself reside. They include sugar, amino acids, minerals, hormones, and even substances which boost immunity to prevent diseases after sprouting. On the other hand, after blastogenesis or germination, seeds consume those ingredients. Additionally, extractives and extracts are acceptable, but real food is favorable to take without extraction or concentration considered from long human eating habits.

The need for assured efficacy with minimized toxicity as food

However, the cost increases when it takes several months to awake the seeds and it is difficult to decide the endpoint of seed awakening by a conventional method. More importantly, the ingredients become uneven if they are not awakened at the same time.

Therefore, a rapid method of awaking the grape seeds synchronizedly was developed as proprietary Grandir recipe^{TM} with 5d of dormancy breaking. At this time, the seed coat was removed and the digestible and absorbable parts of endosperm was obtained.

Here, it is very important to assess rapid and synchronized dormancy-broken grape seed endosperm (RSDB-GSE) in order to assure bioavailability and efficacy with toxicity.

Study objectives

The objective of the in vitro study was to determine the efficacy and toxicity on anti-cancer and pro-immunity of rapid and synchronized dormancy-broken grape seed endosperm (RSDB-GSE) in case of oral administration as food.

Methods (Fig. 1)

Sample

The seeds of Kyoho grape ("giant mountain" grape is tetraploid cross between Ishiharawase (European sp. *Vitis vinifera* × American sp.) and Centennial (*V. vinifera*) produced in Tamushimaru, Kurume, Fukuoka prefecture, Japan and of Niagara grape (*V. labrusca*, cross between Concord and Cassady) produced in Otaru, Hokkaido, Japan were utilized. Rapid and synchlonized dormancy-breaking was performed by our proprietary Grandir recipe™ using Awakening liquid™ (based on concentrated malt extract, edible; O'Atari Inc., Japan). The endosperm parts were separated. Dried endosperm parts were then pulverized by a grinder and sieved to pass 60 mesh. For untreated sample, Kyoho grape seed endosperm was separated without Grandir recipe™. Commercial anti-cancer drugs used were doxorubicin, paclitaxel and gemcitabine.

Methods (Fig. 1)

Digestion and absorption treatment

For the use of oral administration, the endosperm was digested in the stomach step and duodenum step using a human model system including enzymes and bile extract. Then the fraction of molecular weight of 10,000 or less was filtered as an intestinal absorption fraction for assay [10-12].

Methods (Fig. 1)

Anti-cancer (direct-killing) assay

Cell based assay was performed by using our proprietary High Precision-Surface Plasmon Resonance (HP-SPR)-3D system, non-label and –invasive 1h phenotypic screening by mitochondrial membrane potential, as follows [13]. Two-dimensionally cultured viable cells were self-adhered onto an HP-SPR sensor chip, and then collagen was overlaid to obtain in vivo-like cell status. The cell response change was measured for about 1h after the sample addition. The Collagen gel droplet embedded culture drug sensitivity test (CD-DST) was also used as a 3D cell culture method [13, 14]. Cancer cell lines for human were pancreas MIA-PaCa2, breast MCF-7 and liver Hep G2, and that for canine breast was SNP. Normal cell line for human was skin fibroblast HFB16D.

Methods (Fig. 1)

Pro-immunity assay (including indirect-killing for anti-cancer)

Human cell line KHYG-1 was used as natural killer (NK) cells. As target cells, human acute T lymphoblastic cell line CCRF-CEM was hired. Released lactate dehydrogenase was assayed and T lymphocytes cytotoxic activity by NK cells was measured by colorimetric method [15]. Co-culture period was changed into 2.5h. The NK cell proliferation was examined after 2.5h.

Methods (Fig. 1)

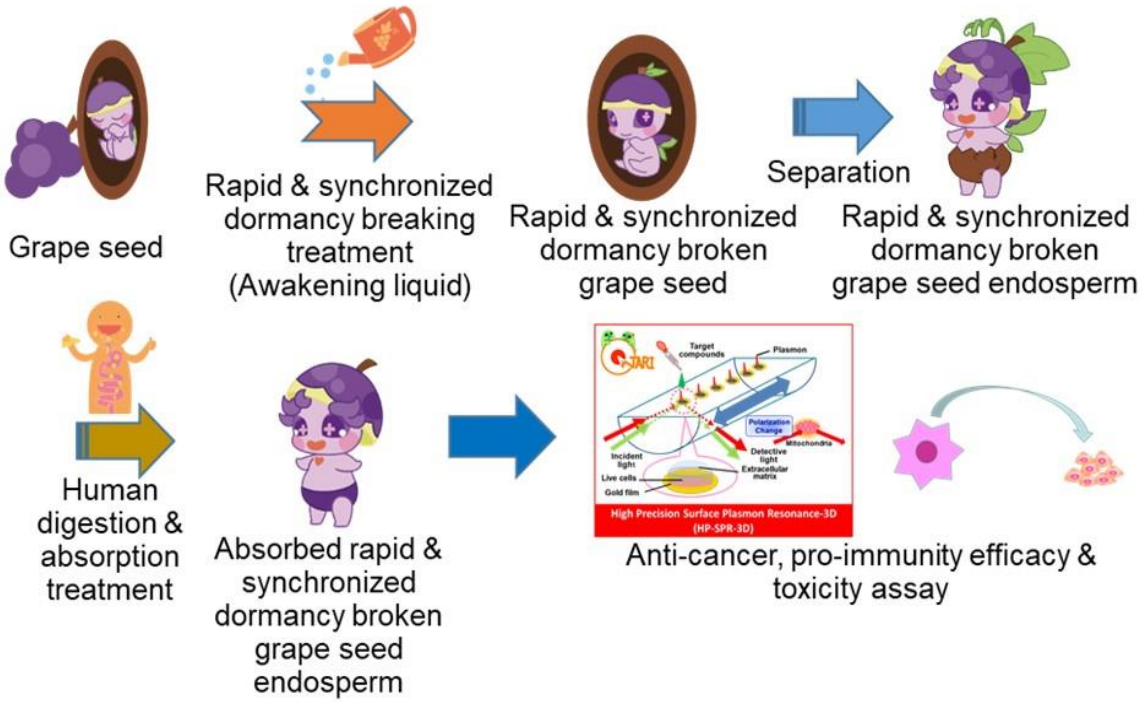


Fig. 1 Experimental scheme.

Results

Anti-cancer (direct-killing) efficacy and toxicity

For human pancreas, anti-cancer (direct-killing) efficacy was observed in a dose-dependent manner at 2.5 mg/ml or more, and it was the maximum at 5.0 mg/ml for RSDB-GSE (Fig. 2). At concentrations higher than this, it showed toxicity of cell functional shutdown different from generally observed necrosis. The reason why the signal of HP-SPR-3D became almost nothing although almost no cell on a sensor chip showed rupture. The efficacy was equivalent to or higher than that of all of tested commercially available anti-cancer drugs used for intravenous administration. Here, the maximum apoptotic efficacy was depicted for all substances.

Results

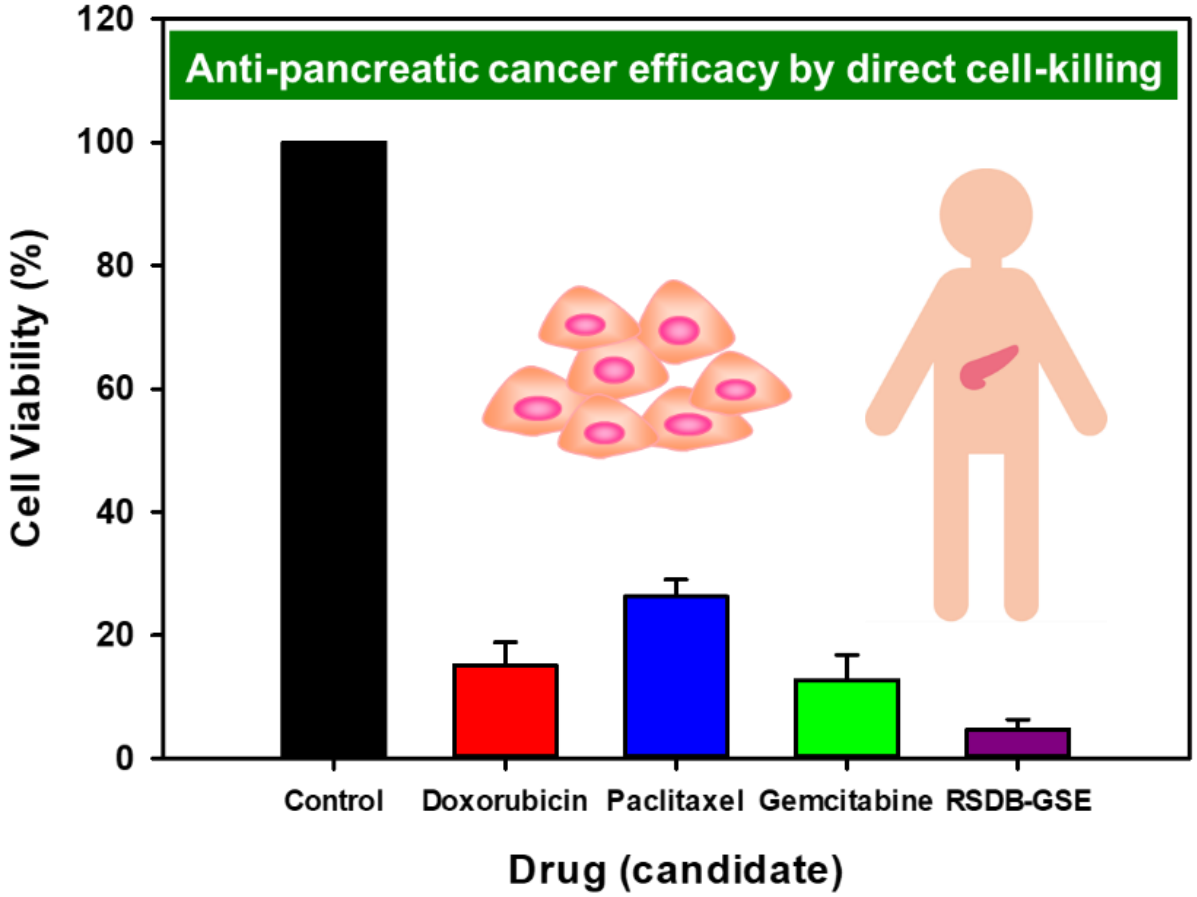


Fig.2 Comparison of anti-pancreatic cancer effect of rapid and synchronized dormancy-broken Kyoho grape seed endosperm (RSDB-GSE) to commercially available anti-cancer drugs against human.

Results

In case of awakened Niagara grape seed endosperm, the anti-pancreas cancer efficacy was about 80%.
For human liver, similar results to pancreas were observed (Fig. 3). Contrast to this, higher concentration was required for human breast (Fig. 4). Other conditions are similar to the pancreas.
On the other hand, RSDB-GSE was also effective against canine breast cancer (Fig. 5).
In case of untreated Kyoho grape seed endosperm, no efficacy was observed at 3.75 mg/ml and necrosis toxicity was started at 5.00 mg/ml against human liver. RSDB-GSE did not work against human normal skin fibroblast at 7.5 mg/ml.

Results

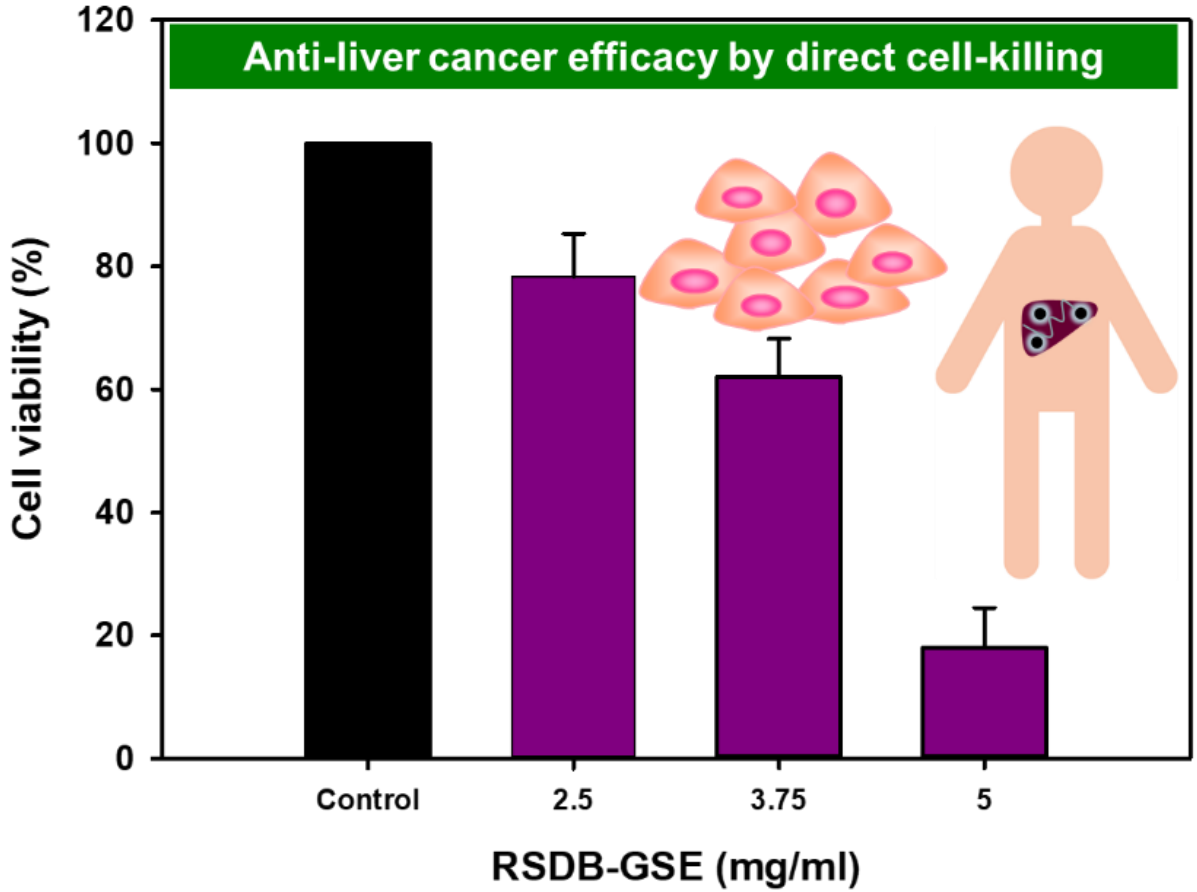


Fig. 3 Anti-liver cancer effect of rapid and synchronized dormancy-broken Kyoho grape seed endpsperm (RSDB-GSE) against human.

Results

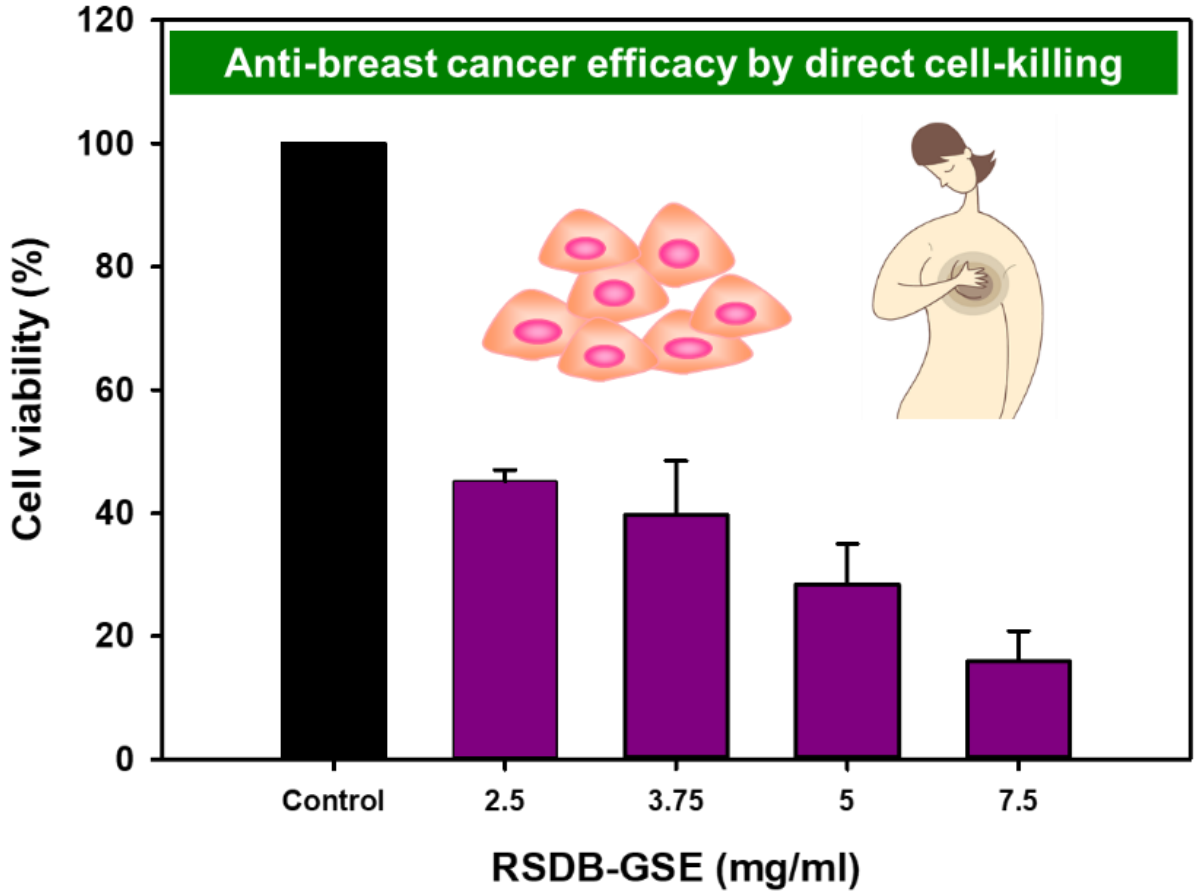


Fig. 4 Anti-breast cancer effect of rapid and synchronized dormancy-broken Kyoho grape seed endosperm (RSDB-GSE) against human.

Results

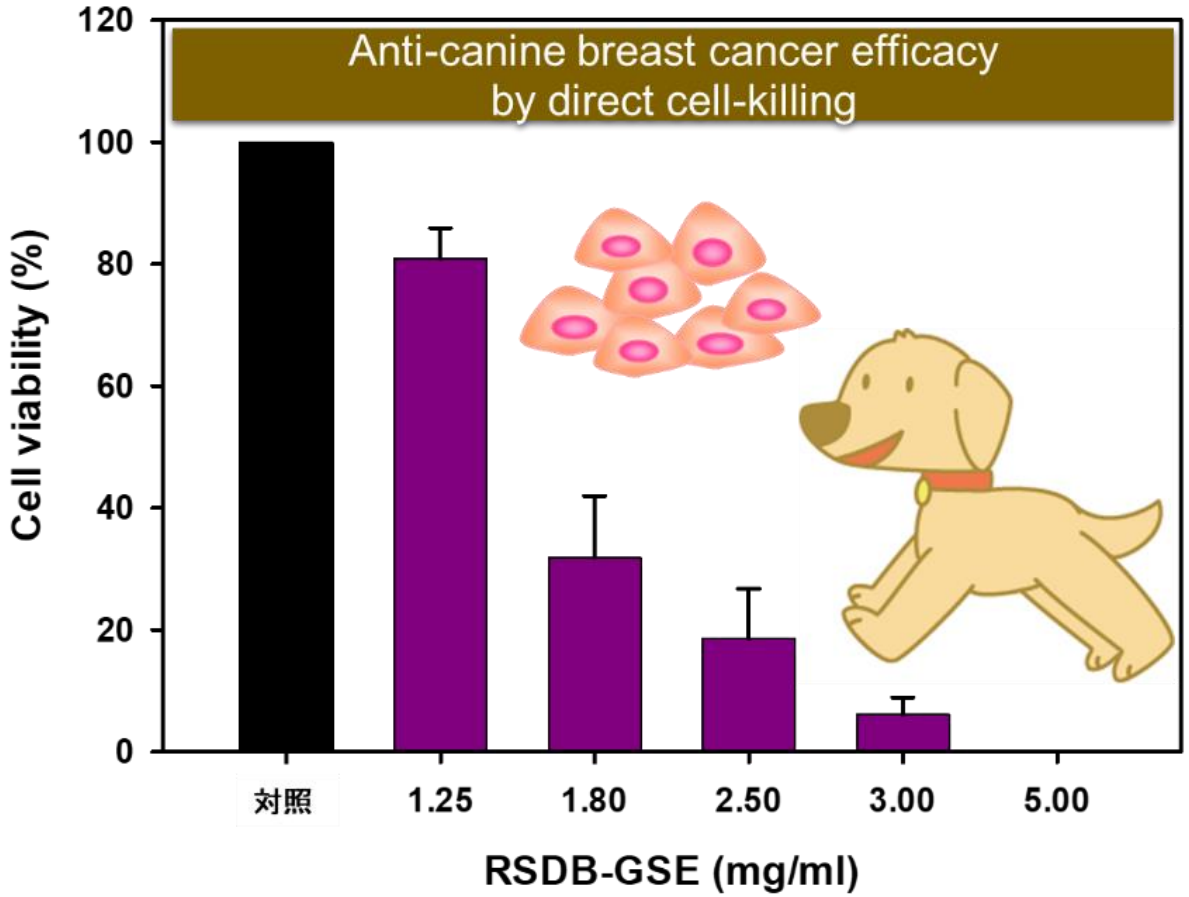


Fig. 5 Anti-breast cancer effect of rapid and synchronized dormancy-broken Kyoho grape seed endosperm (RSDB-GSE) against canine.

Results

T lymphocytes cytotoxic activity by NK cells was observed in a dose-dependent manner at 3.75 mg/ml or more, and it was kept at least until 5.0 mg/ml. Very high pro-immunity was observed after 2.5h (Fig. 6).
The NK cell proliferation was 1.38 times against control only after 2.5h.

Results

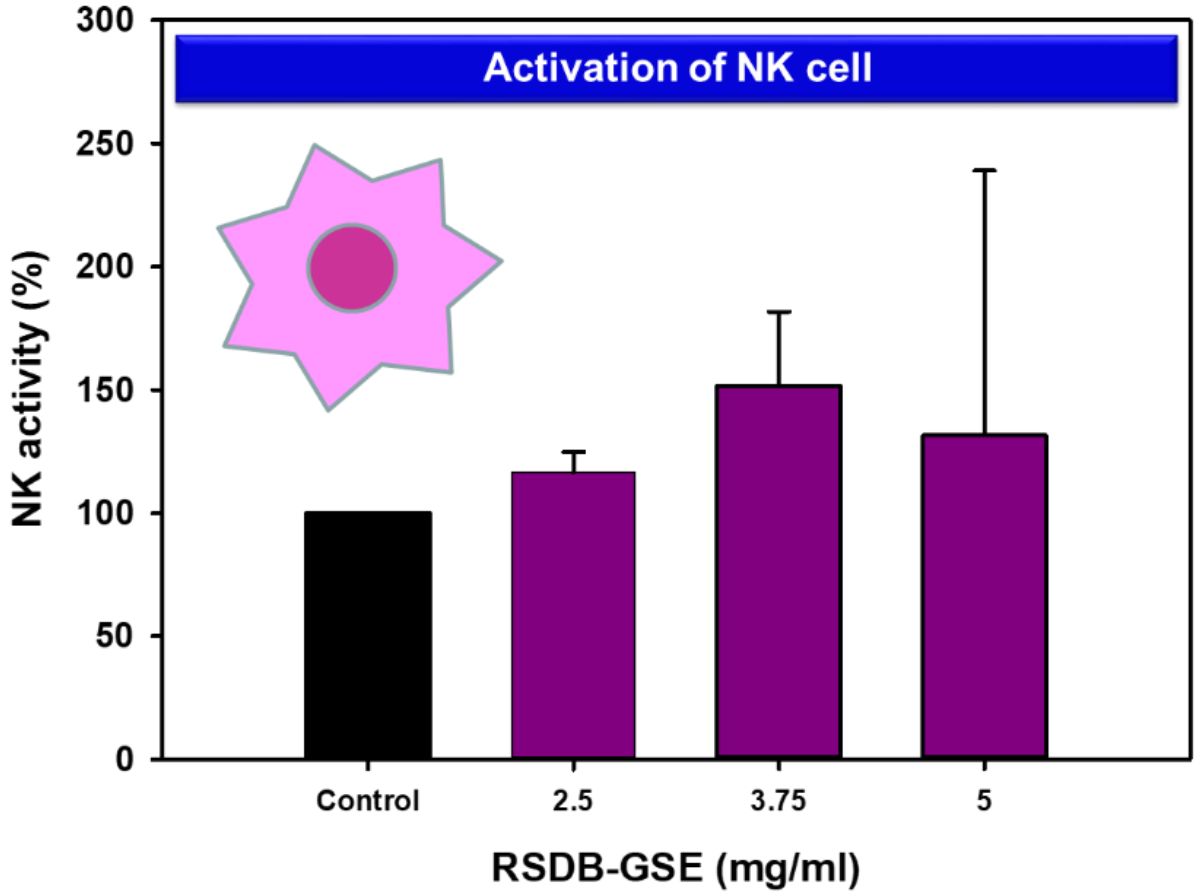


Fig. 6 Pro-immune effect of rapid and synchronized dormancy-broken Kyoho grape seed endosperm (RSDB-GSE) against human NK cell.

Discussion

Although grape seed shows anti-cancer efficacy potential, it possesses toxicity as it is even for extracted active compound [5]. This was conquered by Grandir recipe™ of rapid and synchronized dormancy-breaking technology. The main substances are polysaccharides because the seed coat's removal, different from polyphenol based GSE.

Assuming oral administration, RSDB-GSE showed significant anti-cancer efficacy against pancreas, liver and breast for humans and breast for canines. The efficacy was significantly high similar to commercially available anti-cancer drugs. It possesses both direct efficacy of cancer cell-killing and indirect efficacy of pro-immunity at its similar physiological concentration. Consequently, it is expected to express synergistic effect by these two types of functions (Fig. 7). Here, at least white fruit grape seed will have a similar efficacy because awakened Niagara seed endosperm showed anti-pancreas cancer efficacy. The variety difference might exist for each organ although further study is required to use an available variety.

Discussion

At over dosing, RSDB-GSE showed cell function shutdown, which might cause diarrhea for oral administration although untreated Kyoho grape seed endosperm showed necrosis toxicity. Furthermore, RSDB-GSE showed no efficacy against human normal cell without toxicity at high-dose. The Grandir recipe™ was proven as effective process to increase or generate efficacy with minimizing toxicity. Furthermore, it showed pro-immunity on increase in human NK cell activation and proliferation only after 2.5h. Thus, RSDB-GSE will contribute to health maintenance against infection diseases including corona virus even for healthy people (Fig. 8) [16, 17].

Consequently, rapid and synchronized dormancy-broken Kyoho grape seed endosperm (RSDB-GSE) was successfully developed as effective and safe functional food supplement.

Discussion

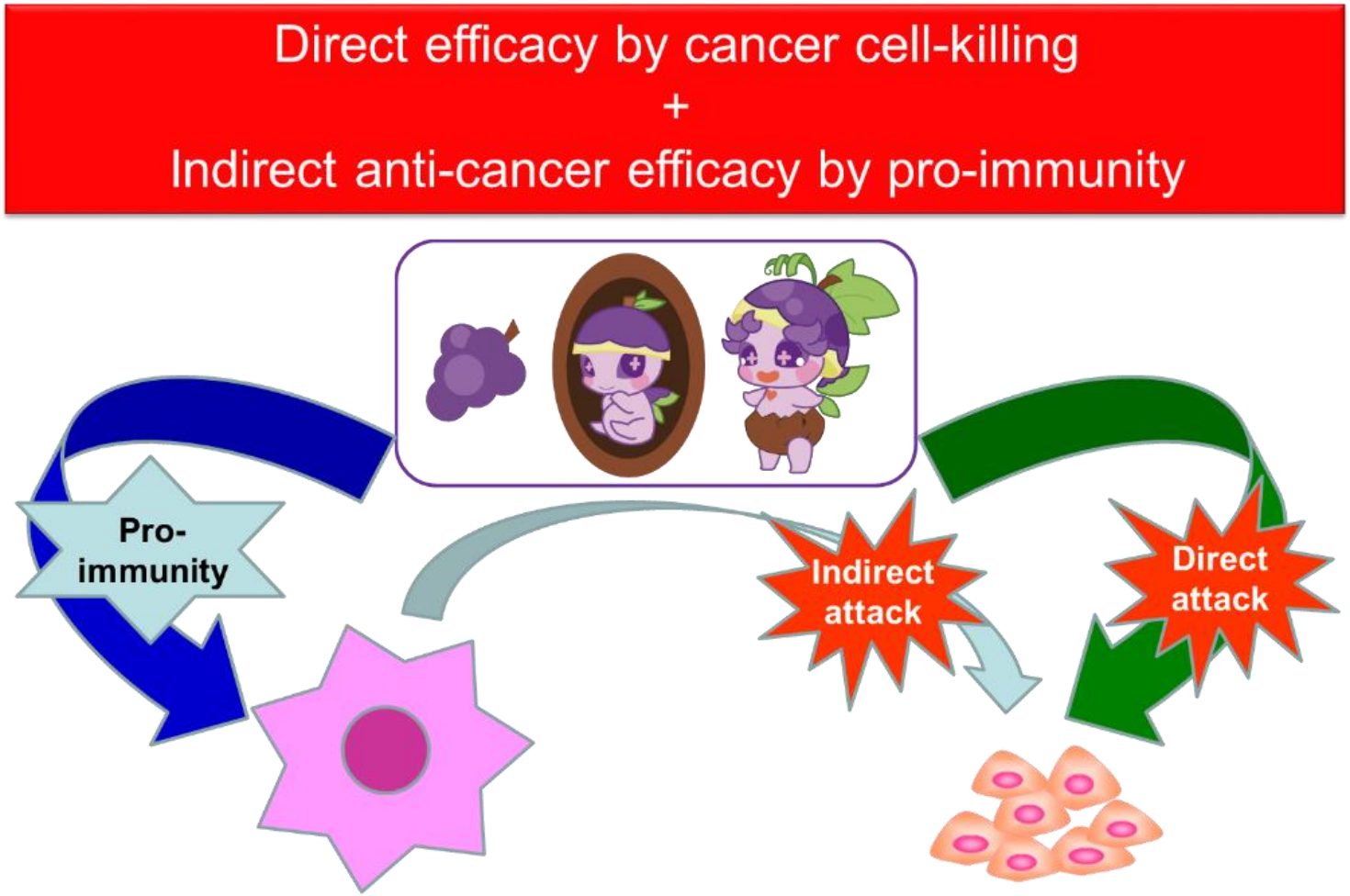


Fig. 7 Synergistic anti-cancer effects of rapid and synchronized dormancy-broken Kyoho grape seed endosperm (RSDB-GSE).

Discussion

Pro-immunity by activation of NK cell
+
Pro-immunity by activation of NK cell proliferation

Fig. 8 Pro-immunity effect of rapid and synchronized dormancy-broken Kyoho grape seed endosperm (RSDB-GSE).

Conclusion

Rapid and synchronized dormancy-broken Kyoho grape seed endosperm (RSDB-GSE) showed two things:

Suitable for therapy and prevention against cancer disease with minimized toxicity by oral administration in view of direct killing and indirect killing efficacies through pro-immunity of NK cell, even synergistically.

Suitable for fight and prevention against infection with minimized toxicity by oral administration in view of pro-immunity on increase in human NK cell activation and proliferation.

Consequently, rapid and synchronized dormancy-broken Kyoho grape seed endosperm (RSDB-GSE) are suitable for functional food supplement.

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Acknowledgments

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