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Differentiation and Apoptosis Induction by Lovastatin and γ-Tocotrienol in HL-60 via Ras/ERK/NF-κB and Ras/Akt/NF-κB Signaling Dependent Down-Regulation of Glyoxalase 1 and HMG-CoA Reductase

Tzou-Chi Huang[1,2*] **and Nelma Nyvonne Tiqu Gina**[2]

[1]Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung, Taiwan

[2]Department of Food Science, National Pingtung University of Science and Technology, Pingtung, Taiwan

[*]**Corresponding author:** Tzou-Chi Huang, Department of Food Science, National Pingtung University of Science and Technology 1, Shuefu Road, Neipu, Pingtung 912, Taiwan, E-mail: tchuang@mail.npust.edu.tw

Introduction

It is well established that cancer cells consume a larger amount of glucose and use a less efficient glycolysis pathway (2 ATP) over mitochondrial oxidative phosphorylation (36 ATP) for quick proliferation [1]. Malignant cells increase glucose uptake and utilization to compensate for the shortage in ATP supply for adaptation to intermittent microenvironmental hypoxia [2]. The suppression of mitochondrial respiration results in the shortage of acetyl-CoA for cholesterol biosynthesis [3]. This article aims to place emphasis on the key survival enzymes for cancer cell survival and proliferation with a multiple target system as a potential therapeutic method for cancer therapy. In addition, progressive research has shown that epigenetics plays a huge role in the underlying mechanisms that lead to cancer cell apoptosis. This article therefore presents a discussion based on the mechanism of epigenetics as an accompanying concept to further deduce our proposal.

Lipid Raft and Cholesterol Synthesis in Cancer Cell Membranes

Lipid rafts are cholesterol- and sphingolipid-enriched microdomains in cell membranes that affect cell survival mechanisms through the regulation of downstream phosphorylation cascades *in vitro* and *in vivo*. Cholesterol is one of the major components of lipid rafts in cancer cell membranes. Our recent publication [4] confirmed that cholesterol homeostasis is abnormal in malignant cells and cholesterol depletion causes cancer apoptosis through differentiation in leukemia cells. Cholesterol biosynthesis is enhanced by the metabolism of glucose through the overexpression of 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), the rate-controlling enzyme (NADH-dependent, EC 1.1.1.88; NADPH-dependent, EC 1.1.1.34) of the mevalonate pathway. Cancer cells utilize glucose via glycolysis to produce ATP for energy requirement. Methylglyoxal (MG) is mainly formed as a by-product of glycolysis. However, over-activated glycolysis pathway forms MG from the triose phosphate intermediates, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate in cancer cells [5,6]. Excess of toxic metabolite methylglyoxal (MG) inhibits cell proliferation by inducing cell cycle arrest and apoptosis. Glyoxalase (GLO1) is the key detoxifying enzyme to eliminate MG and protect cancer cells from apoptosis [7]. A physiological concentration of MG causes nuclear fragmentation and leads to apoptotic cell death in human monocytic leukemia U937 cells [8] and HL-60 cells [9]. MG has a strong cytotoxicity and is able to cross link with protein and DNA to form adducts, resulting in an inhibition of DNA synthesis, stopping cell proliferation and inducing apoptosis [10].

Action of Survival Enzymes and Cholesterol-depleting Agents on HL-60 Cells

We have proceeded to term these two enzymes, HMGCR and GLO1 as survival enzymes for having vital functions in cancer cells as evidenced by our work. Apoptotic activities of lovastatin and γ-tocotrienol were deliberated in HL-60 cells in support of previous studies. Down regulation of the survival enzymes followed by decreased cholesterol synthesis and dissevered lipid raft arose from inhibition of intracellular signalling pathways namely Ras/Raf/ERK/NF-κB and Ras/PI3K/Akt/NF-κB pathways (Figure 1). It is well established that HMGCR activity is up-regulated in some malignant cells compared with their normal counterparts. In addition to leukemic cells, elevated expression of the HMGCR is observed in breast, ovarian and colorectal cancer [11]. Freshly isolated AML and chronic myelogenous leukemia cells display much higher specific HMGCR activity than leukocytes from healthy subjects. The HMGCR activity was 3-fold higher in mononuclear blood cells from patients with leukemia, compared with cells from healthy subjects [12]. The increase of GLO1 expression has been shown to be associated with increased proliferative activity of tumors. It has been shown that GLO1 expression was significantly up-regulated in prostate cancer LNCaP [13], hepatocellular carcinoma [14], and human gastric cancer [15], when compared with adjacent nontumorous tissue. Immunohistochemical analysis has confirmed the increase of GLO1 among patients with prostate cancer [16], and breast tumor [17]. The action of γ-tocotrienol on HL-60 cells was shown to affect the Ras/Raf/ERK/NF-kB/GLO1 and Ras/Akt/NF-kB/GLO1 pathways and thereby demonstrating the execution of activated Ras proteins on cell survival and proliferation [16]. The direct action of γ-tocotrienol on cell death inducers including caspases, Bid-cleavage and Bcl-2 genes has been reported as inducing apoptosis in HL-60 cells [18]. In doing so, over expressed HMGCR is inhibited and influence from the PI3K/Akt pathway is terminated leading to apoptosis as previously reported in neoplastic mammary epithelial cells [19]. An increased phosphorylation of Akt by threonine/serine kinases following signaling of PI3K generates activities such as cell survival and proliferation. Apoptosis is therefore reduced as mentioned in the literature [20]. Here high cholesterol is seen as the major factor of increased phosphorylation in malignant cells. An increased glucose uptake exceeds demand for ATP supply resulting in shortage of acetyl-CoA for cholesterol biosynthesis [3]. Administration of cholesterol synthesis

therefore contributes greatly to recruitment of activated Akt to the cell membrane after being phosphorylated through PI3K signaling. The various observations made in different leukemia samples [21] by the Ras oncogene-triggered pathways can also be seen in HL-60 cells when inhibitors, U0126, LY294002 and JSH-23 were used. Inhibitors U0126 on ERK1/2 and LY294002 on Akt reduced activation of NF-κB through the Ras/Raf/ERK/NF-κB and Ras/Akt/NF-κB pathways respectively. This inactivation of NF-κB was further reduced directly by the inhibitor JSH-23 rendering the translocation of NF-κB to the nucleus inhibited. This influence of γ-tocotrienol on decreased expression of GLO1 as a survival enzyme is significant and showed by results from western blots [4] signifying its apoptotic effects on HL-60 cells. As for the mevalonate pathway it can be deduced that Farnesyltransferase (FTase) is the key enzyme connecting the mevalonate pathway to Ras/Raf/MEK/ERK and PI3K/Akt signaling cascades. Inhibitors of FTase act to prevent Ras from maturing into its biologically active form, and hence FTase, when inhibited by γ-tocotrienol, in turn inactivates mutated Ras proteins. In cholesterol synthesis the mevalonate pathway by far remains the main pathway and a major cholesterol precursor. It is safe to say that increased cholesterol synthesis parallels with the level of over expression of HMGCR enzyme in cancer cells and is in support of previous studies [22,23]. To further support this, HMGCR as a survival enzyme, was being down regulated by treatment with lovastatin. This affects integrity of the cell membrane and in turn decreases cholesterol content which is the major component to stabilize the structure of the lipid raft. Because of this the state of the lipid raft is revisited repeatedly to place emphasis on the strong influence by HMGCR inhibition.

Epigenetic Mechanism and Cancer Cell Apoptosis

Epigenetics refers to a group of heterogeneous processes that regulate transcription without changing the DNA coding sequence, ranging from DNA methylation, to histone tail modifications and transcription factor activity. These changes include acetylation, methylation, phosphorylation and ubiquitination [24].

Figure 1: Schematic model of lipid raft-mediated signalling in the regulation of cell survival and apoptosis in cancer cells during cholesterol depletion treatment. This scheme highlights the importance of lipid raft membrane domains for NF-κB constitutive activation in the regulation of cancer cell survival enzymes and the inhibition of cholesterol biosynthesis leading to the disruption of lipid raft and the subsequent apoptosis.

Histone methyltransferase (HMT) G9a is the main enzyme for dimethylation at Lys 9 of histone H3 to establish H3K9me2 [25]. G9a also stabilizes imprinted DNA methylation in embryonic stem cells by recruitment of de novo DNA methyltransferase enzymes [26]. It has been found that depletion of G9a inhibits cell proliferation in head and neck squamous cell carcinoma [27] and fetal pulmonary artery smooth muscle cell (PASMC) [28]. Recently, Li et al. reported that G9a inhibition induces autophagic cell death via AMPK/mTOR Pathway in Bladder Transitional Cell Carcinoma [29]. Epigenetic regulation of cellular phenotype and proliferation plays a critical role in malignant transformation and tumorigenesis. DNA methylation and histone modifications are the most developed targets for anticancer therapy [30]. Histone methylation modifiers regulate signaling pathways including NF-κB, RAS/RAF/MEK/MAPK, PI3K/Akt/mTOR, Wnt/β-catenin, p53, and ERα. The Ras/Raf/MEK/ERK cascade and its downstream transcription factor targets NF-kB, AP-1, c-Myc and Ets-1 were recognized as proto-oncogenes. Activation of this pathway is commonly observed in malignantly transformed cells [31]. Among them, the Mammalian Target of Rapamycin (mTOR) pathway plays a key role in sensing and integrating multiple environmental signals to regulate cell growth and proliferation [32]. PI3K/AKT/mTOR signaling pathway is essential for the survival of both Primary Effusion Lymphoma (PEL) and Kaposi Sarcoma (KS) [33]. mTOR and its substrate Akt have been isolated from lipid rafts traditionally associated with the plasma membrane [34]. In addition to cholesterol, the mevalonate pathway provides intermediate farnesyl pyrophosphate for RAS protein farnesylation, which is critical for the activation of downstream signaling pathways. Subsequent regulatory processes can stimulate DNA Methyltransferase (DNMT1) activity as well as trigger changes in Histone Deacetylase (HDAC) activity and microRNA levels in various cancer cell lines [35]. The nutri-epigenomic role of n-3 polyunsaturated fatty acids (n-3 PUFA) in relation to colon cancer has been discussed intensively. It is well established that n-3 PUFA regulates signaling processes via the incorporation into cell membranes [36]. The changes in membrane composition affect the structure of lipid rafts and the subsequent intracellular signaling processes [37]. It has been reported that EGFR promotes lung cancer cell formation and proliferation via the Ras/ERK/Myc pathway [38]. In colon cancer cells, Docosahexaenoic acid (DHA, 22: 6n-3) increases the level of Myc protein, an important regulator of cell proliferation, which is believed to induce a chemoprotective, proapoptotic phenotype [39]. NF-kB activity can be inhibited by n-3 PUFA Docosahexaenoic acid (DHA). This is relevant because NF-kB mediates signaling pathways that control the transcriptional activation of genes important for the regulation of many cellular processes and is aberrantly activated in many types of cancer [40]. A disruption of lipid raft by the depletion of cholesterol may downregulate the expression of the three survival enzymes, glyo 1, GCLC and HMGR for cancer cells. As G9a is able to interact with NF-kappaB transcription factor RelB [41] and blocking the function of G9a is sufficient for disturbing P13K/Akt/mTOR pathway [42,43], we postulated that lovastatin and γ-tocotrienol induced cancer apoptosis by influencing the function of G9a.
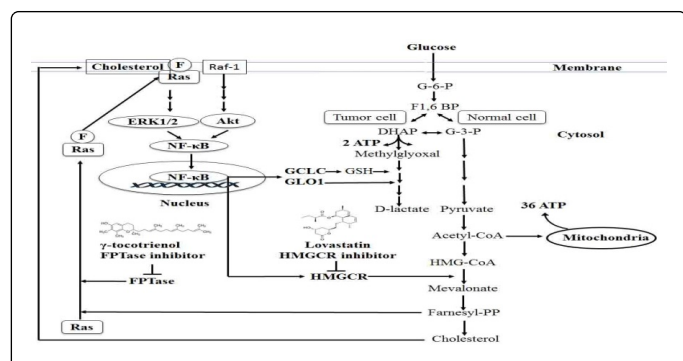
Conclusion

In conclusion, the multiple targets by administration of lovastatin and γ-tocotrienol dose-dependently provides an exceptional example of the functions of natural compounds in HL-60 cells treatment. A combined effect was imparted by the treatment of lovastatin and γ-tocotrienol on HMGCR and FPTase respectively [4]. Lovastatin and γ-tocotrienol act as cholesterol depletion agents to destabilize the lipid raft, interfere with downstream signaling of Ras/Raf/ERK and Ras/PI3K/Akt pathways to NF-kB and suppress the survival enzymes

expression ultimately leading to cancer cell apoptosis. The survival enzymes, HMGCR and GLO1, have been identified here as key survival enzymes for cancer cell survival and proliferation. Considering that they are specifically responsible for cholesterol biosynthesis and as detoxifying enzyme not only do they target HL-60 cells but can be used as a therapeutic basis for various cancer cells.

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