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, mutations in the *SPINK* genes were found to be significantly associated with tumour stages. These findings suggest that *SPINK* gene mutations may be correlated with the tumour stage. Protein and mRNA expression levels of *SPINK* genes [Sec16] ----- All of the *SPINK* proteins were detected in the LSCC by immunohistochemistry. The expression of SPINK3 protein in OSCC was markedly higher than in normal tissues. Both SPINK1 and SPINK3 were strongly expressed in OSCC, and SPINK1 and SPINK3 were highly expressed in the tumour samples of T3 and T4 stages, indicating that SPINK1 and SPINK3 may participate in the pathogenesis of OSCC. The mRNA levels of all the four *SPINK* genes, including *SPINK3*, were upregulated in OSCC. Moreover, the mRNA expression of *SPINK* genes in T1 and T2 stages was higher than in T3 and T4 stages, suggesting the loss of ability to express SPINK1 and SPINK3 in the late stage of tumours. Together, our data suggested that the *SPINK1* and *SPINK3* genes were highly expressed during the early stage of tumour formation and may promote the malignant transformation of LSCC, while the downregulation of *SPINK* genes may occur during the late stages of tumour formation. The SPINK family is mainly found in the oropharynx and exocrine glands [CR15]. However, the expression of the *SPINK* genes in OSCC has not been reported to date. This is the first study to show the highly expression of the *SPINK1* and *SPINK3* genes in OSCC. Furthermore, the results revealed that the expression of *SPINK1* and *SPINK3* mRNA in OSCC was upregulated in the early stage of tumour formation, indicating that they may be involved in the tumour formation, while the expression of *SPINK1* and *SPINK3* mRNA may be lost during the late stage of tumour formation. The main function of SPINK1 is to inhibit the activity of trypsin [CR16]. In addition, the amino acid sequence and 3-D structure of SPINK1 are homologous to those of human plasma kallikrein. SPINK1 is

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In summary, this study demonstrated that EGCG and its precursors possessed anti-MDR activity by increasing ROS, activating caspase-3, and AIF. Increased ROS were attributed to the decreased levels of GSH in non-tumor liver and kidney cells, and were the primary cause for cell death. In tumor cells, this effect was reversed by co-treatment with the antioxidants GSH and NAC. Thus, the use of EGCG and its precursors alone or in combination with anticancer drugs may provide a potential approach for the treatment of MDR cancers, particularly drug-resistant osteosarcoma and breast cancer. 4. Materials and Methods [sec4-molecules-23-03013] ===== 4.1. Reagents [sec4dot1-molecules-23-03013] ----- EGCG (Yong-Yang Biological Technology, Taipei, Taiwan) was dissolved in pure water and kept at -20 °C until used. Lyophilized EGCG was purchased from Kewang Bio-pharmaceutical Ltd. (Zhejiang, China). The purity of EGCG was >95% based on HPLC analysis. NAC (Xiamen Meilun Biological Technology, Xiamen, China) and Cys were purchased from Sigma (St. Louis, MO, USA). GSH and 2,4-dinitrophenylhydrazine (DNPH) were purchased from Merck (Darmstadt, Germany). All other solvents were of HPLC grade and obtained from Merck (Darmstadt, Germany). 4.2. MTT Assay for Cell Viability [sec4dot2-molecules-23-03013] ----- Breast cancer cells were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). MCF-7 and MDA-MB-231 cells were cultured in F12K (ATCC), and hFOB 1.19 cells were cultured in α -MEM (ATCC) at 37 °C in a humidified 5% CO₂- atmosphere. The medium was supplemented with 10% fetal bovine serum (FBS) (ATCC) and 1% antibiotic/antimycotic solution (ATCC). Cell viability was evaluated by the MTT method as described

System Requirements For Photoshop CC 2014:

Graphics: - 4GB of VRAM - DirectX 11.1-compatible card - OS: Windows 7 or Windows 8.1 - 64-bit OS - 1.9 GHz processor or faster DirectX: - Version 11 - Windows 7 or Windows 8.1 Controllers: - DualShock 4 or Xbox One controllers - Headset Optional Configurations: - Single player - 1080p (1280x720) - 30

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