

4-HYDROXYCOUMARIN EFFECTS ON BOTH CELLULAR AND GENETIC CHARACTERISTICS OF HEPATOCELLULAR CARCINOMA CELLS

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4-Hydroxycoumarin is an aromatic substance which is metabolized in liver and used as a therapeutic agent for various diseases. We aimed to determine the impact of 4-Hydroxycoumarin on HepG2 cells according to their viability, proliferation, adhesion and gene expression of cellular behavior parameters. Inhibitory concentration 50 (IC50) of 4-Hydroxycoumarin was detected on HepG2 cells. After determining the optimal time and concentration, the effect of 4-Hydroxycoumarin on viability, proliferation and adhesion of HepG2 cells were observed. Gene expressions of Ki-67, MMP-2, MMP-9 and piR-823 expression were determined by using Real Time-Polymerase Chain Reaction. IC50 value of 4-Hydroxycoumarin on HepG2 cells was 5 μM at the 48th hour (p < 0.001). 5 μM at the 48th hour of 4-Hydroxycoumarin caused to decrease of proliferation (p < 0.001) and viability of HepG2 cells (p < 0.001). Viability rate were supported by hematoxylin-eosin staining. Adhesion of cells increased on 4-Hydroxycoumarin treated cells compared to control (p < 0.001). While Ki-67 gene expression of 4-Hydroxycoumarin treated group decreased (p < 0.001); upregulation of MMP-2, MMP-9 and piR-823 expressions were determined in 4-Hydroxycoumarin treated group (p < 0.001). According to the cellular and genetic perspective, 4-Hydroxycoumarin might be useful to treat hepatocellular carcinoma. High adhesion and proliferation are the main characteristics of HepG2 cells, 4-Hydroxycoumarin treatment caused to lose these functions. The genetic markers of these characteristics also supported the same result. These are first findings about the effect of 4-Hydroxycoumarin on piR-823 and genes which are key features of cellular survival mechanisms.

Key words: 4-Hydroxycoumarin, Matrix metalloproteinase, piRNA, piR-823, Hepatocellular carcinoma

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ВПЛИВ 4-ГІДРОКСИКУМАРИНУ НА КЛІТИННІ ТА ГЕНЕТИЧНІ ХАРАКТЕРИСТИКИ КЛІТИН ГЕПАТОЦЕЛЮЛЯРНОЇ КАРЦИНОМИ

4-гідроксикумарин – це ароматична речовина, яка виробляється в печінці та використовується в якості терапевтичного засобу при багатьох хворобах. Мета нашого дослідження полягала у визначенні впливу 4-гідроксикумарину на клітини HepG2, а саме на їхнє виживання, проліферацію, адгезію, експресію генів, параметри поведінки клітин. Було виявлено інгібіторний вплив концентрації 50 (IC50) 4-гідроксикумарину на клітини HepG2. Після визначення оптимального часу і концентрації спостерігали вплив 4-гідроксикумарину на виживання, проліферацію і адгезію клітин HepG2. Експресію генів Ki-67, MMP-2, MMP-9 і piR-823 визначали за допомогою полімеразної ланцюгової реакції у реальному часі. Значення IC50 4-гідроксикумарину для впливу на клітини HepG2 становило 5 мкМ станом на 48-у годину (p < 0,001). Вплив 5 мкМ 4-гідроксикумарину станом на 48-у годину спричинив зниження проліферації (p < 0,001) і виживання клітин HepG2 (p < 0,001). Рівень виживання визначали за допомогою фарбування гематоксилином-еозином. Рівень адгезії клітин, оброблених 4-гідроксикумарином, підвищився порівняно з контролем (p < 0,001). Рівень експресії гену Ki-67 у групі, обробленій 4-гідроксикумарином, знизився (p < 0,001); позитивна регуляція експресії MMP-2, MMP-9 та piR-823 була встановлена у групі, обробленій 4-гідроксикумарином (p < 0,001). З клітинної і генетичної точки зору 4-гідроксикумарин може бути корисним при лікуванні гепатоцелюлярної карциноми. Високий рівень адгезії і проліферації – це основні характеристики клітин HepG2, застосування 4-гідроксикумарину спричинило втрату цих функцій. Генетичні маркери цих характеристик також підтвердили цей результат. Це перші результати дослідження щодо впливу 4-гідроксикумарину на piR-823 і гени, які є ключовими характеристиками механізмів виживання клітин.

Ключові слова: 4-гідроксикумарин, матрична металопротеїназа, piRNA, piR-823, гепатоцелюлярна карцинома.

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