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Molecular mechanism of anti-cancerous potential of Morin extracted from mulberry in Hela cells

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ABSTRACT

The flavonoids are of great interest due to their antioxidant and anti-cancerous potential. The present study investigated the Morin extracted from mulberry leaves and its anti-cancerous mechanism via cell inhibition, cell cycle analysis, apoptosis, mRNA expression and antioxidant mechanism through reactive oxygen species generation. Morin inhibited the proliferation of HeLa cells at IC50 of 214.28 μM and led to morphological changes, followed by induction of cell cycle arrest in G2/M-phase and ultimately resulted into apoptosis. Morin-induced G2/M-phase arrest was accompanied by the increase in mRNA expression of p53, p21 and Wee 1 genes and decreased levels of CDK1, Cdc25c, Survivin, cyclin B1and CHK2. Morin-induced apoptosis was regulated through multiple pathways, including intrinsic and extrinsic pathway. The underlying mechanisms consisted of increased mRNA expression of Bax, Bad, cytochrome c, Apaf-1, caspases-9, DR3, DR5, FasL, FADD, caspases-10, PARP, PI3K, AKT, mTOR, P70S6K and Smac genes as well as decreased expression of Bcl-2, Bcl-xL, AMPK, cIAP-1, cIAP-2, PKCε and NF-kβ. In addition, Morin treatment resulted in the generation of intracellular ROS, which play an essential role in the induction of apoptosis. The present study recommends the use of Morin in the development of functional foods with anti-cancerous potential in the future.

1. Introduction

In the recent years, various cancer has been one of the leading causes of mortality (Jin et al., 2014b). Among various cancers, cervical cancer is placed at second most common malignant tumor in women worldwide (Eskander and Tewari, 2014; Zhang et al., 2017c) with particular reference to developing nations due to inadequate health care infrastructure and non-sufficient screening programs. Though, the standard care therapy constituting chemotherapeutic drug (cisplatin) and surgery is available along with their significant side effects (Knoff et al., 2014). Therefore, the researchers functioning on cancer prevention and its cure are drawing their attention towards exploring novel and effective natural agents which can reduce the onset of cervical cancer and deal with the increasing incidences of this particular disease. However, studies constituting their underlying molecular mechanism for anti-cancerous activities still remain unclear.

Numerous naturally occurring substances exert their anti-cancerous activity by arresting cell cycle and triggering tumor cell apoptosis which appear to be the best strategy to combat abnormal cell growth (Dewanjee et al., 2017). Apoptosis is basically a programmed cell death accompanied by morphological changes such as membrane bubbling, cell shrinkage, chromatin condensation, nuclear fragmentation, and chromosomal DNA fragmentation (Song et al., 2012) which are regulated at cell signaling pathways levels. Alteration of these apoptotic pathways cause many pathological conditions such as autoimmune diseases, cancer as well as viral infections (Torkzadeh-Mahani et al., 2012). Previous studies demonstrated the number of techniques (flow cytometry with annexinV-FITC/propidium iodide (PI) staining) to detect the early stages of apoptosis depict that under normal conditions, most phosphatidylserine (PS) molecules are localized at the inner layer of the plasma membrane, but in the early apoptotic cells, PS re-distributes to the outer layer of the membrane and gets exposed to the extracellular environment (Sano et al., 2012).

On the other hand, mitochondria, typically the center of the

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respiratory chain and oxidative phosphorylation (Song et al., 2012), acts as an important regulator for promoting or inhibiting programmed cell death through intrinsic pathway as well as an important source of ROS during apoptosis (Gupta et al., 2012). The loss of the mitochondrial membrane potential ultimately leads to an increased generation of ROS and apoptosis (Na et al., 2008). ROS generation is one of the important mechanisms for induction of apoptosis, which obviously blocks the migration of cancer cells, induce cell cycle arrest and triggers the apoptosis (Droge, 2002), DNA damage and genomic instability which cause tumorigenesis. In mammalian cells, cell cycle is tightly controlled by the G1, S, and G2/M phase checkpoints and cyclin dependent kinases (CDKs) play an important role in the cell cycle. It is well known that many compounds affect cyclin and/or CDKs and, thus, may lead to cell cycle arrest. NF-kappa Beta is a transcription factor which participates in inflammation, immunity, and oncogenesis (Shin et al., 2017). The mitogen-activated protein kinase (MAPK) and AKT pathways have been identified as the key signaling pathway involved in cancer progression, thus, targeting them would be an effective therapeutic selection for controlling cell proliferation and cell migration (Shin et al., 2017). The mitogen-activated protein kinase (MAPK) and AKT pathways have been identified as the key signaling pathway involved in cancer progression, thus, targeting them would be an effective therapeutic selection for controlling cell proliferation and cell migration (Shin et al., 2017).

In the past years, some small molecular compounds, such as millepachine and piperlongumine, have been reported to induce apoptosis through the generation of ROS and inhibit the growth of malignant breast cancer and as well as their associated migration in mice (Raj et al., 2011). Therefore, development of new and effective natural agents for the up regulating the level of ROS can pave the way for treating different types of malignancies.

Since the various phytochemicals are known to modulate the cancer cell biology and induce the death of abnormally growing cancer cells (Surh, 2004), considerable interest has been drawn to utilize and make the most use of these plant-derived active factors for preventing or controlling cancer. Previously, plant components such as ginger and polysaccharides have been known to possess anti-cancerous attributes (Zhang et al., 2017a, 2017b). Particularly, flavonoids as plant pigments in dietary plants, root and components of herbal-containing dietary supplements (Zhang et al., 2011) have drawn much attention in recent years due to array of biological activities that possess the biological actions, such as anti-cancerous (Sivaramakrishnan and Niranjali Devaraj, 2009), anti-inflammatory (Fang et al., 2003a) and anti-fibrotic activities. Moreover, the intake of fruits and vegetables is associated with cancer prevention (Hatcher et al., 2008) and thus, it is rational to explore various kinds of flavonoids for anti-cancerous effects preventing.

Morin as a flavonoid is present in fruits, vegetables, tea, wine, and many oriental medicinal herbs and mulberry figs and old fustic (Chung et al., 2016). Morin has been reported to possess several biological and biochemical effects including potent antioxidant, anti-inflammatory, anti-cancerous, anti-neoplastic, cardio protective activities, anti-hyperuricemia (Brown et al., 2003b; Zhang et al., 2016; Fang et al., 2003b) and anti-tumor effect in liver cancer (Hyun et al., 2015a; Sivaramakrishnan et al., 2008), breast cancer (Jin et al., 2014a), colon cancer (Karthik et al., 2009) and oral tumor (Brown et al., 2003a). Moreover, some researches had reported that Morin could inhibit the cancer cell targeting various mechanisms (Hyun et al., 2015a,b; Li et al., 2016; Shin et al., 2017; Karimi et al., 2013; Thomas et al., 2016). Prevents the destruction of β-cells in streptozotocin-induced diabetic rats thereby exert its anti-hyperglycemic effect (Vanitha et al., 2014, 2017). The antioxidant potential of Morin is due to the scavenging superoxide anions and highly reactive species which play significant role in the initiation of lipid peroxidation (Li et al., 2016; Zhang et al., 2016). Vanitha et al. (2017) investigated the anti-oxidative potential of Morin which activated the Nrf2-ARE pathway and thereby reduced the oxidative stress -induced DNA damage in pancreatic beta cells (Vanitha et al., 2017). Morin acts as potent anti-oxidant and shield against oxidative damages through modulation of metabolic enzymes, including cytochrome P450 and protect various human cells, like myocytes, endothelial cells, hepatocytes and erythrocytes (Naso et al., 2013). Due to the beneficial effects, previous studies have predicted that Morin could be exploited under *in vitro* and in vivo cancer models to validate its anti-cancerous potential (Jin et al., 2014b; Subash and Subramanian, 2008). However, few studies have reported the use of Morin on cancer cell growth and metastasis, but its mechanisms of the anti-cancerous activity were not well studied in Human Cervical Carcinoma Hela cells.

To the best of our knowledge, our study is the first ever report for anti-cancerous attributes of Morin on Hela cells. Hence, the essential objective of this study was to investigate the molecular mechanism of Morin-induced cell cycle arrest and apoptosis in Human Cervical Carcinoma Hela cells. In brief, we investigated the effects of Morin on the cell proliferation, morphology, cell cycle, apoptosis, and also on the generation of reactive oxygen species (ROS). Furthermore, the expression of genes related to cell cycle and apoptosis was also evaluated. Throughout the course of this study we observed that Morin has significant anti-cancerous potential, through which it could inhibit the cancer cells growth and their proliferation which could lead to cell-cycle arrest and apoptosis.

2. Materials and methods

2.1. Materials

The mulberry leaves were procured from the mulberry fields of Anhui Sericultural Research Institution (Hefei, China). The cell line growth media ingredients (Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), L-glutamine, penicillin-streptomycin and 0.25% trypsin solution without EDTA) were procured from Invitrogen (Carlsbad, USA). The cancer cell model (Hela cells) used in our study was Human cervical cancer cell line obtained from Shanghai Wei atlas biological technology co., LTD.

2.2. Morin extraction and HPLC analysis

The Morin was extracted from mulberry leaves followed by purification for high performance liquid chromatography (HPLC) by following the procedures given in the previous studies (Hussain et al., 2014; Shi et al., 2012). In brief, the air-dried grounded mulberry plant material was exhaustively extracted with 100% methanol in a shaking incubator at room temperature for 24 h. As per the previous study, HPLC analysis was performed on a Shimadzu LC-8A system, equipped with a 515HPLC pump, a 20 μL injection loop, and an SPD-m10AVP UV detector with system controller SCL-10AVP. UV detection was done at 280 nm and 340 nm. The purity of Morin was determined as 98.81% (Supplementary Fig. 1).

2.3. Cell culture conditions

Before beginning the treatment with Morin, Hela cells were placed into 60 mm × 15 mm culture dishes and grown in DMEM high glucose medium supplemented with10% heat-inactivated FBS, 2 mM L-glutamine, and 1% penicillin-streptomycin. The cells were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 and used for further experimentation.

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