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Non-conventional rottlerin anticancer properties

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ABSTRACT

In the past few years, we focused the interest on rottlerin, an old/new natural substance that, over the time, has revealed a number of cellular and molecular targets, all potentially implicated in the fight against cancer. Past and recent literature well demonstrated that rottlerin is an inhibitor of enzymes, transcription factors and signaling molecules that control cancer cell life and death. Although the rottlerin anticancer activity has been mainly ascribed to apoptosis and/or autophagy induction, recent findings unveiled the existence of additional mechanisms of toxicity. The major novelties highlighted in this mini review are the ability to bind and inhibit key molecules, such as ERK and mTOR, directly, thus independently of upstream signaling cascades, and to cause a profound dysregulation of cap-dependent protein translation through the mTORC1/4EBP1/eIF4E axis and by inhibition of eIF2, an initiation factor of translation that is negatively regulated by endoplasmic reticulum (ER) stress. These last mechanisms, proved to be lethal in cancer cell lines derived from breast and skin, strongly enforce the potential of rottlerin as a promising natural lead compound for the development of novel therapeutic approaches.

1. Introduction

The plant kingdom is a rich source of several clinically useful anticancer agents. Most of currently available drugs are indeed natural compounds or synthetic derivatives, structurally related to them (epipodophyllotoxin lignans, taxane diterpenoids, camptothecin quinolone and the vinca alkaloids) [1].

A wide range of additional natural compounds, present in edible plants or extracted from herbal medicine, are known to exhibit anticancer activities, not only in "in vitro" but also in "in vivo" model, by interfering with multiple aberrant signaling pathways. Emblematic examples are garlic [2], green tea [3], red wine [4], turmeric [5] and soy [6]. Most of them exert their effects by regulating key signaling molecules that control cancer cell growth, inflammation and apoptosis.

There is currently much interest in the identification and development of chemotherapeutics that act on specific molecular and cellular targets, since a proper understanding of the mechanisms by which they operate is needed to establish their efficacy.

Usually, genetic and molecular alterations are responsible for aberrant cancer cell growth. By contrast, normal cells have normal and balanced signaling pathways and therefore should better resist to targeted chemotherapy. It follows that directing the therapy against the oncogenic molecules could be the most selective strategy. However, because most cancer cells bear more than one alteration, mono-target drugs are often inefficient to eradicate the tumor and might even lead to selection of resistant phenotypes. Therefore, it is currently universally held that the so-called combination therapy is the best therapeutic approach.

1.1. The rottlerin history

Rottlerin (E)-1-[6-[(3-acetyl-2,4,6-trihydroxy-5-methylphenyl)methyl]-5,7-dihydroxy-2,2-dimethylchromen-8-yl]-3-phenylprop-2-en-1-one), molecular weight of circa 516 g/mol, is the principal component of kamala, the red powder that covers the fruits of the Mallotus philippinensis, an evergreen tree that grows in the tropical regions of Southeast Asia (Fig. 1). Since ancient times, kamala is used in folk medicine to cure infective/inflammatory ailments, such as scabies, bronchitis, abdominal disease and others [7]. The kamala powder is also efficacious against tape-worm, probably because of its laxative effect [8] and/or thanks to the rottlerin ability to activate Big potassium (BK) channels that hyperpolarize cell membranes [9,10], thereby

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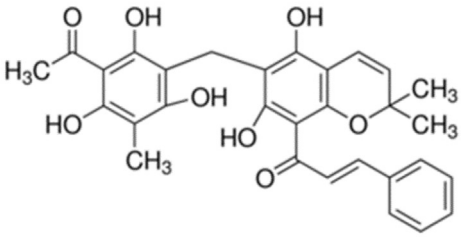
Mallotus philippinensis

ripe fruit

kamala powder



rottlerin structure

Fig. 1. Rottlerin sources and chemical structure.

paralyzing the parasite buccal apparatus necessary for attachment to the intestine of the host.

The fruit of the Mallotus tree is not edible and rottlerin must be extracted from the powder kamala. However, the total synthesis of rottlerin has been recently described and its biological activity is under evaluation [11].

Rottlerin has been used for decades as a selective PKCδ inhibitor [12] although some controversy on this aspect still exists. In recent years, the research revealed several other properties of rottlerin, such as oxidant quencher [13], antiproliferative [14], antiangiogenic [15], anti-inflammatory [16], anti-allergic [17] anti-microbial [18], anti-fungal [19] and anti-parasitic [20].

As far as cancer is concerned, several studies have reported rottlerin efficacy against different tumor cells and through multiple mechanisms of action. Rottlerin indeed can be now considered a multitarget drug, able to interfere with both the apoptotic and the autophagic pathways and thus to exert, alone or in combination therapies, a successful anticancer action [21]. The ability of rottlerin to interfere with breast cancer cells and melanoma cells proliferation have been extensively investigate by our and other groups and in this review, we would like to summarize the state of the art on these particular cancer models.

1.2. Rottlerin and breast cancer

Since the first isolation in the '70s [22], one of the most widely used "in vitro" models for breast cancer are the MCF-7 cells, which indeed has served as a fundamental model for the study of anticancer agents.

In addition, the suggested use of MFC-7 breast cancer cells is supported also by the fact that this cell line has a high apoptotic threshold due to caspase 3 deletion and Bcl-2 overexpression. Furthermore, MFC-7 cells express low Beclin-1 levels, hence making MCF-7 also resistant to autophagy triggered by canonical pathways.

In the study by Torricelli et al. [23], we found that rottlerin not only inhibits proliferation, as previously reported [14], but also kills MCF-7 cells, time- and dose-dependently, in not starved conditions and in the absence of any other treatment. This study presented evidence that the death mechanisms triggered by rottlerin in MCF-7 cells are determined by the functional availability of caspase-3, since the death mode switched from autophagy, in caspase 3-deficient cells, to apoptosis in caspase 3-tranfected cells.

The main conclusion from this study was that rottlerin could be cytotoxic for different cancer cell types, in both ways, either triggering autophagy in apoptosis-resistant cells or stimulating apoptosis in apoptosis-competent cells.

The investigation on the mechanistic aspects of cell death revealed that, while the apoptotic process in caspase 3-tranfected MCF-7 cells followed the classical mitochondrial pathway (Bcl-2 downregulation, caspase- 9 and -3 activation), the autophagic death, in caspase 3-deficient MCF-7 cells, being Bcl-2-, Beclin 1-, Akt- and ERK- independent, appeared to be triggered by non-canonical signaling pathways [23].

A subsequent study revealed that rottlerin caused MCF-7 cells autophagic death by mTORC1 inhibition but, again, this occurred by an unusual mechanism that was independent from phosphorylation events and occurred without recruitment of known signaling molecules upstream of mTOR, such as AMPK. By using rottlerin-coupled Sepharose beads (the beat) to "capture" mTOR (the prey) into the cellular extract (pull-down assay), it was found that rottlerin inhibited mTOR by forming a stable complex with the protein.

The main result from this study was the identification of a novel mechanism by which rottlerin can modulate signaling molecules: the ability to inhibit key enzymes, such as mTOR, by direct interaction (binding).

1.3. Rottlerin and melanoma

In addition to MCF-7 cells, the cytotoxic effects of rottlerin have been also evaluated in other cancer cells with different backgrounds and chemosensitivity. In this regards it is worth to mention the recent work on melanoma cells in which Sk-Mel-28 melanoma cells were treated with rottlerin. As for MCF-7, which are apoptosis resistant due to the loss of the caspase-3 gene and overexpression of Bcl-2 protein, SK-Mel-28 cells bear a number of genetic alterations that makes them refractory to most chemotherapeutics. This cell line indeed, in addition to the presence of B-RAF V600E variant, mutant p53, and PTEN [24], is characterized by the down-regulation of the p21/Cip1 gene [25] and overexpression of cyclin D1, resulting from genomic amplification [26].