Polyphenols-rich extract from *Araucaria angustifolia*: Differential mechanisms on cancer and normal cells

Cátia dos Santos Branco¹, Tiago Selau Rodrigues¹, Émilin Dreher de Lima¹, Mirian Salvador¹

¹Laboratório de Estresse Oxidativo e Antioxidantes; Instituto de Biotecnologia, Universidade de Caxias do Sul, RS 95070560, Brazil

Correspondence: Mirian Salvador
E-mail: msalvado@ucs.br
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Cancer cells present differential metabolism compared to normal cells. Multiple molecular mechanisms converge to alter cellular metabolism, and some of these include a process of metabolic reprogramming which provides advantages to tumor cells in energy generation, growth and proliferation. Tumor energy production is basically dependent on glucose driven to glycolysis (Warburg effect), but it also happens by means of fatty acids and glutamine metabolism. Among the current challenges in cancer therapy, the tumor cell resistance and the absence of selectivity of anti-cancer agents stand out. It has been already shown that polyphenols are able to exert differential effects on normal and tumor cells. However, the exact mechanisms of these actions are not fully understood. In our previous study, we showed that a polyphenols-rich extract (PE) from *Araucaria angustifolia* held a selective capacity to inhibit the proliferation of human larynx HEp-2 cancer cells with minimal cytotoxicity to normal epithelial cells. We hypothesized that the effect presented by PE have happened by reversing the “Warburg effect” on cancer cells and inhibiting the mitochondrial electron transport chain complex I activity along with ATP depletion on these cells. In this research highlight we will discuss the effects of the PE on mitochondrial metabolism and their possible role in the modulation of mitochondrial sirtuin 3 (SIRT3) on tumor (HEp-2) and normal (HEK-293) cells, which may help to clarify the tumor selectivity exhibited by polyphenols.

**Keywords:** Cancer; mitochondria; redox status; SIRT3; polyphenols

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For example, paclitaxel has good antiproliferative effects on human breast cancer MCF-7 cell; however, it is able to damage normal cell seriously, as shown by Zhao et al.\[11\]. In this sense, natural or chemical compounds that display selective cellular targets are promising candidates in the anti-cancer therapeutics research.

Due to the central role of mitochondrial activity in critical cellular processes, such as metabolism and apoptosis, mitochondria have been considered an important target for cancer therapy \[12-14\]. Mitochondrion is an important bioenergetics organelle of the cell. Besides ATP generation and control of redox homeostasis, it also modulates calcium flux throughout the cell and mediates the intrinsic pathway of apoptosis \[15-17\]. Mitochondrial function depends on their assembly, maintenance and dynamics, and the occurrence of disruptions in this balance may cause primary and secondary mitochondrial dysfunction, being hence deleterious to the cell \[16\]. Typically, the primary dysfunction is associated with mutations to nuclear genes encoding mitochondrial proteins, whereas secondary dysfunction is generally caused by events from other etiology \[16, 18, 19\]. Often, the dysfunction of mitochondria is related with calcium homeostasis disruption, deficient ATP generation and enhanced reactive oxygen species (ROS) formation, mainly through the electron transport chain (ETC), which induces the formation of the mitochondrial permeability transition pore (MPTP) leading to mitochondrial damage \[16, 20\]. The complex I of ETC, also namely NADH: ubiquinone oxidoreductase is among the major sites of superoxide anion radical (O$_2^-$) generation \[20\], one of the most common form of ROS. Superoxide anion radical can be dismutated by the action of the superoxide dismutase (SOD) antioxidant enzyme, producing hydrogen peroxide. Mitochondria contain their own SOD, specifically MnSOD, found into the mitochondrial matrix \[20, 21\], however, high levels of ROS as well as other toxic stimuli, can trigger the intrinsic apoptosis process, which is a
desirable effect in cancer therapy. Intrinsic apoptosis, mediated by mitochondria, is initiated with the release of cytochrome c (Cyt c) into the cytosol, which occurs often as result of mitochondrial membrane permeabilization induced by MPTP formation, and lead to initiation of the apoptotic process and cell death [22]. In this context, mitochondria restoration is an interesting strategy to be explored in cancer research field [23].

Recently, our research group showed that a polyphenols-rich extract (PE) derived from natural source (Araucaria angustifolia) generates high cytotoxicity in human larynx HEp-2 cancer cells, with minimal effect to normal HEK-293 cells [24]. This extract contains several polyphenols belonging to the flavonoids class, including catechin and epicatechin (flavan-3-ol subclass); rutin and quercetin (flavonol subclass); apigenin (flavone subclass); 4'-methoxytectorigenin (isoflavone); 3-glucoside-dihydroquercetin (dihydroflavonol subclass), and the biflavonoid amentoflavone 4",4\(^{-}\),7",7\(^{-}\)-tetramethylether [24-26]. All of these are phenolic compounds that hold recognized anti-cancer properties (for review, see Asensi et al. [27]). We found that the PE inhibited the activity of the complex I of the ETC, and consequently depleted ATP production on cancer cells, leading the cell to death via apoptosis. In contrast, PE administration did not reduce ATP levels on normal cells, although it had minimally diminished complex I activity, and consequently the cell viability was maintained (Figure 1). Considering these data we showed that PE had the ability to alter the metabolism of cancer cells, reversing the Warburg effect and then restoring mitochondrial function in these cells. We postulated that this effect is possible due to the inhibition of the pyruvate kinase isoenzyme M2 (PKM2) on tumor HEp-2 cells, which is a key regulator of the glycolytic pathway, reprogramming the flux of glucose that supplies the metabolic demand of growing cells [28]. This enzyme is one of the four isoforms of pyruvate kinase [29], and acts as a transcriptional co-factor of hypoxia-inducible factor-lalpha, which upregulates pyruvate dehydrogenase kinase 1, causing an inhibition of the pyruvate dehydrogenase [30]. The silencing of PKM2 allows the formation of pyruvate and its conversion to acetil-CoA, consequently activating cellular respiration. Although PKM2 is encountered in few types of normal cells [28, 31], it is present at high levels in tumor cells [3], being an important target for cancer therapy.

The inhibition of mitochondrial complex I by PE showed in our work generated high levels of ROS and ATP depletion, starting the activation of mitochondrial death pathway, through upregulation of Bax expression. Bax proteins translocate from the cytosol to the mitochondrion, facilitating Cyt c and AIF release. Escape of Cyt c is associated with upregulation of cleaved caspases-3, which cleave nuclear proteins, such as PARP, leading to the irreversible process of apoptosis [24].

In our study [24], PE presented minimal cytotoxicity in HEK-293, possibly through the differential modulation on a group of proteins named sirtuins. Sirtuins activity have been implicated in numerous biological processes including cancer, and are messenger molecules that mediate the metabolic status of the cell in response to stress conditions [32]. Mitochondrial sirtuins (SIRT3, SIRT4, SIRT5) are able to regulate cellular metabolism via post-translational modifications [33], and specifically SIRT3 is responsible for coordinating the maintenance of mitochondria in order to produce energy, mediating the oxidative metabolism and redox homoeostasis [34-36]. Moreover, SIRT3 activates the ETC by deacetylating NDUFA9, a necessary component of electron transport complex I, and it is also involved with the maintenance of ATP levels [34, 37]. The expression of Cyt c, another important component of the ETC, is also dependent of SIRT3 [38], as well as the activation of MnSOD antioxidant enzyme [39, 40]. Therefore, we hypothesized that the data shown in our previous study in cancer cells [24] is due to PE downregulation and/or inhibition of SIRT3, resulting in decreased antioxidant response and increased ROS, thus inhibiting ETC and cell growth. On the other hand, in normal cells the antioxidant response was maintained, preventing the cells from oxidative damage and cell death (Figure 1). Similar mechanism was shown for (-)-epigallocatechin-3-gallate, a compound similar to those found in our PE, which was able to suppress the expression and activity of SIRT3 on cancer cells and to increase SIRT3 levels on normal cells [41]. These experimental and hypothetical data evince the need to perform further studies to understand the differential mechanisms of the polyphenols on cancer and on normal cells, which could contribute to find selective targets in cancer treatment.

Conflicts of Interest

The authors declare no conflict of interest.

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References


18. Murphy E, Steenbergen C. What makes the mitochondria a killer? Can we condition them to be less destructive? Biochim Biophys Acta 2011;1813:1302-1308.


