Green tea catechins and the levels of *miR-125b-5p* in MCF-7 breast cancer cells

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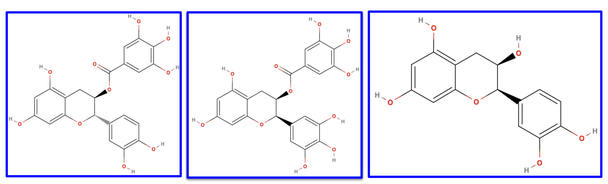
ABSTRACT

Epicatechin (EC) or catechin hydrate (CH), epigallocatechin gallate (EGCG), and catechin gallate (CG) are organic compounds found in green tea known as polyphenolic catechins that have proapoptotic effects on breast cancer cells. Certain microRNAs (miRNAs), small non coding RNA molecules responsible for regulating gene expression, such as *miR-125b-5p*, have also been shown to promote apoptosis in breast cancer cells. Resveratrol, another polyphenolic compound, promoted apoptosis by downregulating expression levels of *miR-125b-5p* in MCF-7 breast cancer cells in previous research. Our study tested the hypothesis that exposure to 25μM and 50μM CH, CG and EGCG will also decrease *miR-125b-5p* levels in MCF-7 breast cancer cells. We used reverse transcription and relative qPCR for our experiment. Our results suggested that CH and EGCG downregulate the expression of *miR-125b-5p* while CG upregulates its expression. As a result, we concluded that CH and EGCG are most likely to promote apoptosis by downregulating expression levels of *miR-125b-5p*. Overall, this study presented innovative research on the relationship between green tea catechins and the levels of *miR-125b-5p* in MCF-7 breast cancer cells.

INTRODUCTION

- Breast cancer is one of the leading causes of death for women worldwide. In 2018 alone, the incidence rate of female breast cancer was 11.6% with 2,088,849 new cases and the mortality rate was 6.6% with 626,679 resulting deaths. [1]
- Several epidemiological studies have suggested that the lower risk of breast cancer in Asian-Americans, could be attributed to their consumption of green tea. [2,3]
- Major components of green tea are polyphenolic catechins including catechin hydrate (CH), catechin gallate (CG), and epigallocatechin gallate (EGCG). [4-7]



Catechin gallate | Epigallocatechin-3- gallate | Catechin hydrate

Figure 1. Structures of different catechins. Retrieved from MolView

- Studies have shown that these catechins can induce apoptosis, programmed cell death, in MCF-7 directly or indirectly by inhibiting the mechanisms that would allow cancerous cells to rapidly proliferate. [8, 5-7]
- However, the mechanisms through which catechins lead to apoptosis in these cells are not known.
- MicroRNAs are small non coding RNA molecules that regulate gene expression in cells throughout the body. [9]
- Studies have shown that miRNA expression becomes deregulated in breast cancer cells. *miR-125b*, for example, is one of the 29 miRNAs that researchers identified as having significantly different expression levels between normal and cancer tissues. [10,11]
- Studies have shown *miR-125b* is involved in promoting apoptosis. [15]

UCCCUGAGACCCUAACUUGUGA

Figure 2. Sequence of *miR-125b-5p*.

- Studies have also shown that resveratrol, a polyphenol, has the ability to alter miRNA expression and particularly lowers the expression *miR-125b*. [15]
- It is not known whether catechins such as EGCG, CH, and CG have the ability to modify *miR-125b* expression levels, and in this way promote apoptosis

We hypothesize that the polyphenolic catechins EGCG, CH, and CG will decrease the expression of *miR-125b-5p*

METHODS

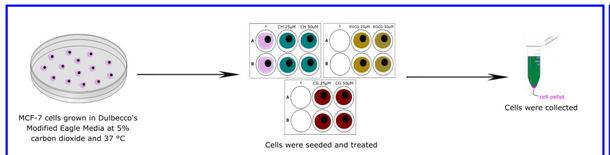


FIGURE 3. Treatment of MCF-7 cells with Catechin hydrate (CH), Epigallocatechin Gallate (EGCG), and Catechin Gallate (CG). Cells were treated with 25μM and 50μM of CH, EGCG, and CG. Duplicate treatments were done for each catechin. Untreated cells were used as control.

FIGURE 4. RNA Extraction. Total RNA was extracted from the treated and untreated cells using Direct-zol RNA extraction kit (Zymo Research). [19]

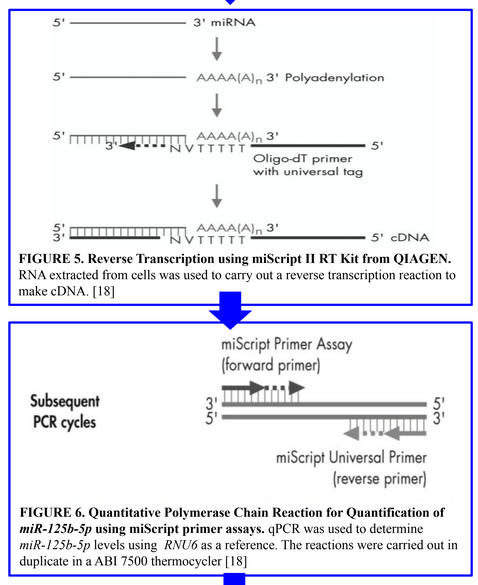


FIGURE 5. Reverse Transcription using miScript II RT Kit from QIAGEN. RNA extracted from cells was used to carry out a reverse transcription reaction to make cDNA. [18]

FIGURE 6. Quantitative Polymerase Chain Reaction for Quantification of *miR-125b-5p* using miScript primer assays. qPCR was used to determine *miR-125b-5p* levels using *RNU6* as a reference. The reactions were carried out in duplicate in a ABI 7500 thermocycler [18]

$$\Delta\Delta CT = (C_{T,miR-125b-5p} - C_{T,RNU6})\ Exposure\ 25\mu M\ or\ 50\mu M - (C_{T,miR-125b-5p} - C_{T,RNU6})\ Exposure\ 0$$

FIGURE 7. Equation for Analysis of qPCR by Relative Quantification. Relative *miR-125b-5p* expression was analyzed using the 2-ΔΔCT method, which allows for the analysis of the expression of a target miRNA to be compared between various samples. *RNU6* was used as the target miRNA. [17]

RESULTS

Table 1. Relative expression of *miR-125b-5p* after 48-hour exposure to Epigallocatechin gallate, Catechin hydrate, and Catechin gallate.

Treatment	25μM	50μM
Catechin gallate	118.21 ± 75.81%	169.79 ± 74.74%
Catechin hydrate	63.59 ± 21.35%	78.79 ± 15.70%
Epigallocatechin gallate	61.98 ± 11.09%	61.22 ± 17.24%

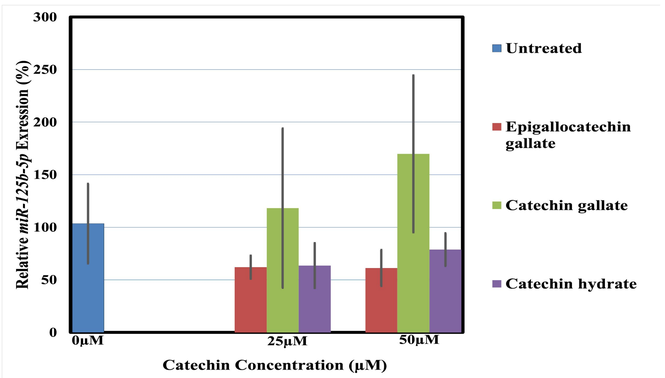


Figure 8. Relative expression of *miR-125b-5p* after 48-hour exposure to Epigallocatechin gallate (EGCG), Catechin hydrate (CH), and Catechin gallate (CG) in MCF-7 breast cancer cells with RNU6 as reference gene. Average relative *miR-125b-5p* expression in MCF-7 cells treated with EGCG, CH, and CG at 25μM and 50μM. Two experiments were conducted for each treatment.

CONCLUSIONS

Summary Of Results:

- Exposure to EGCG and CH at both concentrations resulted in the downregulation of *miR-125b-5p* expression when compared to the control.
  - MCF-7 cells treated with 25μM EGCG showed a relative *miR-125b-5p* expression of 61.98 ± 11.09%. Cells treated with 50μM EGCG showed a relative *miR-125b-5p* expression of 61.22 ± 17.24%
  - MCF-7 cells treated with 25μM CH showed a relative *miR-125b-5p* expression of 63.59 ± 21.35%. Cells treated with 50μM CH showed a relative *miR-125b-5p* expression of 78.79 ± 15.70%
- By contrast, exposure to CG at both concentrations resulted in the upregulation of *miR-125b-5p* expression when compared to the control.
  - MCF-7 cells treated with 25μM CG showed a relative *miR-125b-5p* expression of 118.21 ± 75.81%. Cells treated with 50μM CG showed a relative *miR-125b-5p* expression of 169.79 ± 74.74%.

Conclusions:

- Exposure to EGCG did not change across different concentrations, exposure to CG and CH increased as the concentration of the catechins increased
- Treatment with EGCG and CH decreases levels of *miR-125b-5p*
  - Previous studies have shown that exposure to resveratrol leads to loss of *miR-125b-5p* and promotes apoptosis [15].
  - EGCG and CH could decrease levels of *miR-125b-5p* and potentially induce apoptosis.
- Interestingly, treatment with CG increases the levels of *miR-125b-5p.*
  - In previous research CG promoted apoptosis at similar levels as did CH [20].
  - Higher levels of *miR-125b-5p* would not induce apoptosis so CG must promote apoptosis by other mechanisms that do not involve *miR-125b-5p*.

Limitations:

- Catechins were tested at only two concentrations of 25μM and 50μM and only for one 48-hour exposure time.

Future Research:

- To carry out the experiments at higher concentrations and increase treatment times.

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