**An inulin-type fructan CP-A from *Codonopsis pilosula* alleviated** **5-fluorouracil-induced intestinal mucositis via** **E****RK/MLCK/MLC2 pathway and regulation of gut microbiota**

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**Table S1** Sequences of the primers used in the RT-PCR

|  |  |  |
| --- | --- | --- |
| Gene | Primer | Gene sequence (5’-3’) |
| IL-4 | Forward | ACA AGG AAC ACC ACG GAG AAC G |
| Reverse | TCT TCA AGC ACG GAG GTA CAT CAC |
| IL-6 | Forward | GCC TTC TTG GGA CTG ATG TTG TTG |
| Reverse | GTC TGT TGT GGG TGG TAT CCT CTG |
| IL-10 | Forward | AAG GCA GTG GAG CAG GTG AAG |
| Reverse | TGA GTA TCA CGT AGG CTT CTA TGC |
| TNF-α | Forward | CCG AGA TGT GGA ACT GGC AGA G |
| Reverse | CCA CGA GCA GGA ATG AGA AGA GG |
| ERK1/2 | Forward | GTT CCC AAA CGC TGA CTC CAA AG |
| Reverse | GCC AGA GCC TGT TCA ACT TCA ATC |
| MLCK | Forward | TTC AAG ATG GTG GTG GCT GTG G |
| Reverse | TGC TTG CTC CTT GTT CTC CTC AG |

**Table S2** Relative abundance of the most representative phyla in experimental rats

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Phylum | Control | 5-FU | BTC | CP-A L | CP-A M | CP-A H | Variation (5-FU) |
|  | Relative abundance (%) |
| Firmicutes | 89.49±2.13\*\* | 62.86±12.86 | 80.66±3.20\* | 85.25±4.69\* | 86.32±2.08\*\* | 89.90±2.01\*\* | ¯ |
| Proteobacteria | 2.00±0.14\*\* | 27.03±11.78 | 7.58±3.80\* | 6.71±3.02\* | 4.50±1.14\*\* | 1.67±0.21\*\* | ­↑ |
| Bacteroidetes | 6.97±1.74 | 6.54±2.54 | 9.95±2.23 | 3.24±1.29 | 5.26±2.09 | 6.63±1.83 | ­↑ |
| Actinobacteria | 0.67±0.17 | 2.57±0.94 | 1.01±0.65 | 3.93±1.34 | 2.64±0.19 | 1.15±0.60 | ­↑ |
| Tenericutes | 0.16±0.10 | 0.22±0.19 | 0.06±0.01 | 0.29±0.11 | 0.41±0.23 | 0.12±0.07 | ­↑ |
| Cyanobacteria | 0.17±0.02 | 0.16±0.02 | 0.15±0.01 | 0.16±0.02 | 0.18±0.03 | 0.11±0.01 | ¯ |
| Verrucomicrobia | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 | 0.03±0.02 | 0.06±0.02 | 0.01±0.01 | ¯ |
| TM7 | 0.01±0.01 | 0.00±0.00 | 0.00±0.00 | 0.07±0.06 | 0.02±0.01 | 0.01±0.00 | ¯ |
| Elusimicrobia | 0.01±0.01 | 0.00±0.00 | 0.00±0.00 | 0.07±0.06\* | 0.02±0.01 | 0.01±0.00 | ¯ |

Values were represented the mean±SEM.

Kruskal-Wallis test was applied for statistics and \**p* < 0.05 and \*\**p* *<* 0.01 versus 5-FU group (n = 6).

**Table S3** Relative abundance of the most representative classes in experimental rats

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phylum | Class | Control | 5-FU | BTC | CP-A L | CP-A M | CP-A H | Variation (5-FU) |
| Relative abundance (%)　 |
| Firmicutes | Clostridia | 35.97±3.48 | 43.28±6.54 | 27.71±8.12 | 44.58±9.58 | 46.50±9.99 | 24.43±2.61 | ­↑ |
|  | Bacilli | 50.23±5.06\*\* | 13.38±5.85 | 50.28±10.24\*\* | 37.41±12.35\* | 30.32±7.72 | 61.39±4.20\*\* | ¯ |
|  | Erysipelotrichi | 2.86±1.12 | 5.97±1.91 | 2.39±0.85 | 3.01±1.28 | 9.23±2.67 | 3.81±2.52 | ­↑ |
| Actinobacteria | Coriobacteriia | 0.31±0.04\* | 1.99±0.89 | 0.89±0.66 | 1.18±0.53 | 0.85±0.19 | 0.57±0.29 | ­↑ |
|  | Actinobacteria | 0.35±0.15 | 0.57±0.49 | 0.12±0.03 | 2.75±1.45\* | 1.79±0.22 | 0.57±0.33 | ­↑ |
| Proteobacteria | Alphaproteobacteria | 0.43±0.08\*\* | 0.22±0.02 | 0.24±0.02 | 0.28±0.03 | 0.32±0.04 | 0.21±0.01 | ¯ |
|  | Betaproteobacteria | 0.42±0.04 | 0.25±0.03 | 0.28±0.03 | 0.52±0.21 | 0.37±0.06 | 0.18±0.02 | ¯ |
|  | Gammaproteobacteria | 0.59±0.08\*\* | 25.44±11.94 | 6.03±3.42\* | 1.95±0.63\*\* | 2.47±1.37\*\* | 0.74±0.14\*\* | ­↑ |
|  | Deltaproteobacteria | 0.53±0.14 | 0.99±0.28 | 0.95±0.33 | 3.92±2.44 | 1.06±0.33 | 0.47±0.14 | ­↑ |
| Bacteroidetes | Bacteroidia | 6.96±1.74 | 6.53±2.54 | 9.95±2.23 | 3.23±1.29 | 5.25±2.09 | 6.60±1.83 | ¯ |

Values were represented the mean±SEM.

Kruskal-Wallis test was applied for statistics and \**p* < 0.05 and \*\**p* *<* 0.01 versus 5-FU group (n = 6).

**Table S4** Relative abundance of the most representative orders in experimental rats

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phylum | Order | Control | 5-FU | BTC | CP-A L | CP-A M | CP-A H | Variation (5-FU) |
|  Relative abundance (%) |
| Firmicutes | Clostridiales | 35.97±3.48 | 43.27±6.54 | 27.70±8.12 | 44.58±9.58 | 46.49±9.99 | 24.43±2.61 | ­↑ |
|  | Lactobacillales | 50.07±5.08\*\* | 12.96±5.68 | 50.22±10.23\*\* | 37.23±12.36\* | 29.97±7.64 | 60.60±4.82\*\* | ¯ |
|  | Erysipelotrichales | 2.86±1.12 | 5.97±1.91 | 2.39±0.85 | 3.01±1.28 | 9.23±2.67 | 3.81±2.52 | ­↑ |
| Proteobacteria | Desulfovibrionales | 0.52±0.14 | 0.99±0.28 | 0.95±0.33 | 3.92±2.44 | 1.05±0.33 | 0.47±0.14 | ­↑ |
|  | Burkholderiales | 0.42±0.04 | 0.25±0.03 | 0.28±0.03 | 0.51±0.21 | 0.37±0.06 | 0.18±0.02 | ¯ |
|  | Enterobacteriales | 0.27±0.08\*\* | 25.11±11.93 | 5.79±3.43\* | 1.33±0.69\*\* | 2.20±1.38\*\* | 0.45±0.16\*\* | ­↑ |
|  | Pseudomonadales | 0.28±0.02 | 0.23±0.03 | 0.20±0.03 | 0.57±0.33 | 0.21±0.04 | 0.22±0.04 | ¯ |
| Actinobacteria | Coriobacteriales | 0.31±0.04 | 1.99±0.89 | 0.89±0.66 | 1.18±0.53 | 0.85±0.19 | 0.57±0.29 | ­↑ |
|  | Actinomycetales | 0.32±0.16 | 0.50±0.45 | 0.07±0.01 | 2.71±1.45\* | 1.09±0.36 | 0.38±0.30 | ­↑ |
| Bacteroidetes | Bacteroidales | 6.96±1.74 | 6.53±2.54 | 9.95±2.23 | 3.23±1.29 | 5.25±2.09 | 6.60±1.83 | ¯ |

Values were represented the mean±SEM.

Kruskal-Wallis test was applied for statistics and \*P<0.05 and \*\**p* *<* 0.01 versus 5-FU group (n = 6).

**Table S5** Relative abundance of the most representative families in experimental rats

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phylum | Family | Control | 5-FU | BTC | CP-A L | CP-A M | CP-A H | Variation (5-FU) |
| Relative abundance (%) |
| Firmicutes | Lactobacillaceae | 49.29±5.19\*\* | 11.60±5.33 | 49.90±10.17\*\* | 33.05±13.36\* | 27.86±7.54 | 59.44±5.59\*\* | ¯ |
|  | S24-7 | 4.82±0.88 | 3.43±1.25 | 7.23±1.78\* | 1.97±0.53 | 3.77±1.26 | 5.45±1.54 | ¯ |
|  | Peptostreptococcaceae | 2.84±0.97 | 4.52±2.36 | 2.32±0.97 | 5.73±1.55 | 3.70±1.09 | 2.39±0.63 | ­↑ |
|  | Clostridiaceae | 1.04±0.28 | 1.29±0.56 | 0.36±0.06 | 0.74±0.20 | 0.64±0.14 | 0.53±0.13 | ­↑ |
|  | Erysipelotrichaceae | 2.86±1.12 | 5.97±1.91 | 2.39±0.85 | 3.01±1.28 | 9.23±2.67 | 3.81±2.52 | ­↑ |
|  | Ruminococcaceae | 16.33±2.24 | 8.27±3.28 | 7.56±2.20 | 16.81±4.27 | 16.60±7.39 | 7.40±1.77 | ¯ |
|  | Lachnospiraceae | 8.78±2.44\* | 23.20±6.25 | 11.74±5.17 | 11.55±4.13 | 17.21±5.44 | 9.08±1.16\* | ­↑ |
| Proteobacteria | Enterobacteriaceae | 0.27±0.08\*\* | 25.11±11.93 | 5.79±3.43\* | 1.33±0.69\*\* | 2.20±1.38\*\* | 0.45±0.16\*\* | ­↑ |
|  | Desulfovibrionaceae | 0.52±0.14 | 0.99±0.28 | 0.94±0.33 | 3.92±2.44 | 1.05±0.33 | 0.47±0.14 | ­↑ |
| Actinobacteria | Coriobacteriaceae | 0.31±0.04\*\* | 1.99±0.89 | 0.89±0.66 | 1.18±0.53 | 0.85±0.19 | 0.57±0.29 | ­↑ |

Values were represented the mean±SEM.

Kruskal-Wallis test was applied for statistics and \**p* < 0.05 and \*\**p* *<* 0.01 versus 5-FU group (n = 6).

**Table S6** Relative abundance of the most representative families in experimental rats

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phylum | Genus | Control | 5-FU | BTC | CP-A L | CP-A M | CP-A H | Variation (5-FU) |
| Relative abundance (%) |
| Firmicutes | Lactobacillus | 48.89±5.22\*\* | 11.34±5.21 | 49.42±10.08\*\* | 32.92±13.33 | 27.66±7.49 | 59.21±5.60\*\* | ¯ |
|  | Oscillospira | 0.60±0.22 | 2.45±1.71 | 0.77±0.40 | 3.21±1.37 | 1.17±0.25 | 0.84±0.47 | ­↑ |
|  | Blautia | 4.19±1.69 | 10.69±5.44 | 3.06±0.89\* | 1.84±0.58\* | 4.68±1.70 | 3.72±0.97 | ­↑ |
|  | Dorea | 0.42±0.20 | 2.35±0.79 | 2.25±1.77 | 1.53±1.05 | 0.54±0.26 | 0.29±0.08 | ­↑ |
|  | Ruminococcaceae\_Ruminococcus | 1.79±0.73\* | 0.39±0.16 | 1.22±0.48 | 0.69±0.25 | 0.77±0.18 | 0.87±0.26 | ¯ |
|  | Subdoligranulum | 2.30±1.93 | 0.44±0.23 | 1.08±1.00 | 1.71±1.01 | 1.54±1.18 | 0.62±0.32 | ¯ |
| Bacteroidetes | Bacteroides | 0.10±0.03\* | 1.88±1.23 | 0.78±0.27 | 0.47±0.36 | 0.86±0.62 | 0.25±0.03 | ­↑ |
| Proteobacteria | Shigella | 0.16±0.04\*\* | 24.25±11.64 | 5.45±3.33\* | 1.13±0.67\*\* | 2.00±1.34\*\* | 0.36±0.16\*\* | ­↑ |
|  | Desulfovibrio | 0.45±0.13 | 0.79±0.22 | 0.67±0.19 | 2.33±1.17\* | 0.91±0.29 | 0.38±0.12 | ­↑ |
| Tenericutes | Allobaculum | 2.21±1.14 | 5.60±1.82 | 1.59±0.86 | 2.36±1.36 | 8.54±2.80 | 2.74±2.64 | ­↑ |

Values were represented the mean±SEM.

Kruskal-Wallis test was applied for statistics and \**p* < 0.05 and \*\**p* *<* 0.01 versus 5-FU group (n = 6).