

From genetic diversity to bioactive effects: the Bowman-Birk protease inhibitor.

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Abstract: The need of bioactive compounds naturally occurring in food is raising awareness among the researchers. In this context, a potential legume component, the Bowman-Birk protease inhibitor (BBI), was evaluated as a chemopreventive agent against colorectal cancer (CRC). One hundred and one accessions of *Vigna unguiculata* from Africa were genetically screened to explore the natural biodiversity of the gene encoding for the BBI. Fifteen different haplotypes corresponding to the seven existing protein isoforms were found, some of them with peculiar mutations in the interactive loops. Then, the most common isoform was identified in a commercial lot, which extracts were previously demonstrated to be bioactive against CRC cell lines but not on the healthy line (Panzeri et al., 2020). The BBI was extracted and purified until a sufficient level of purity through affinity and subsequently size exclusion chromatography. Soybean commercial BBI was selected as standard through all experiments and compared to purified BBI from *Vigna unguiculata*. To confirm the identity of the BBI, trypsin inhibition assays were performed. Soybean BBI showed a higher inhibition ability, by about 20% in comparison with *V. unguiculata* BBI ($p < 0.05$). Then, the two BBIs plus aqueous extract containing the BBI from the previous reference paper were tested on three CRC cell lines (one healthy and two from tumors) at increasing concentrations. As shown before, the extracts were able to decrease CRC lines viabilities ($p < 0.05$) while not having any effects on the healthy cell lines ($p > 0.05$). The BBIs though showed a similar behaviour. Both the BBIs showed a dose-dependent effect on all the selected lines while *V. unguiculata* BBI was more effective on the healthy line ($p < 0.05$) and on the DiFi line at 20 $\mu\text{g/mL}$ ($p < 0.05$) in comparison with the soybean BBI. The extract was not effective on the healthy line as expected, suggesting on one hand a potential protective component of the extract itself, on the other hand a trace contaminant in the purified BBI. Further works are needed to confirm the two hypotheses.

Bibliography:

Panzeri, D., Guzzetti, L., Sacco, G., Tedeschi, G., Nonnis, S., Airolidi, C., ... & Regonesi, M. E. (2020). Effectiveness of *Vigna unguiculata* seed extracts in preventing colorectal cancer. *Food & Function*, 11(7), 5853-5865.