

# Bilitranslocase and anthocyanins role in the gastrointestinal tract

Jovana Čvorović, Federica Tramer

LP-University of Trieste

**Abstract** —Bilitranslocase (BTL) is a organic anion transporter expressed in liver and in several extra-hepatic tissues. This membrane protein transports different substrates as pyrrolic molecules, nucleotides, flavonoids. Anthocyanins, one of the most represented flavonoids class are known to have antioxidant activity. They act as anti-inflammatory, anti-cancer and anti-proliferative molecules by interfering with different intracellular pathways. Some chronic colon diseases results in an increase in the pro-inflammatory machinery that are associated with a 5-fold increased risk of developing colon cancer. The expression and the role of BTL in the gastrointestinal tract in normal and pathological condition, as well as its role as anthocyanins transporter in colon cancer cells will be take into account in Trans2care project.

**Index Terms** — bilitranslocase, organin transporter, flavonoids, anthocyanins, colon cancer, chronic colon diseases

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## 1 INTRODUCTION

### 1.1 Bilitranslocase (BTL)

**B**ilitranslocase (BTL) is a plasma membrane transporter of 340 amino acids that displays no homology with known proteins [1]. However, its coding sequence shares 96% identity with a segment of the antisense strand of ceruloplasmin. BTL was originally identified in rat liver, and subsequently found also in absorptive (stomach and intestine) and excretory (kidney) epithelia. Its sub-cellular localization is suitable for the uptake of its substrates from the gastro-intestinal lumen, suggesting its nutritional role. Concerning BTL expression in pathological conditions, preliminary data show reduction in its expression in kidney cancer cells. BTL has also been detected in colon cancer cell line Caco-2 where the transport activity is strongly

inhibited by anti-bilitranslocase antibodies [2]. However, there are no available data on the presence of this transporter in chronic colon diseases.

Bilitranslocase transports various polyaromatic compounds such as bromosulphophthalein (BSP) and indocyanine green, dyes used in the evaluation of hepatic function, but also bilirubin and nucleotides. BTL presence in the stomach [3] with the functional properties identical to those in the liver [4] might explain why flavonoids, as dietary anthocyanins for example, are absorbed a few minutes after their ingestion [5]. On the other hand, BTL high turnover might explain why flavonoids are rapidly metabolized and excreted. The detection of BTL on the luminal surface of the intestinal epithelium could also speak in favor of its role in flavonoid uptake since they are absorbed at this level as well.

## 1.2 Anthocyanins and chronic bowel diseases

Flavonoids are a large group of phytonutrients that may provide beneficial effects on human health. They are divided into several classes: flavanones, flavones, flavonols, flavanols and anthocyanins. The main dietary sources of flavonoids are fruits, vegetables and grains. Epidemiological, clinical and experimental studies have indicated that dietary intake of flavonoids confers protection against multiple chronic diseases [6]. Their possible role in cancer prevention and progression has gained increased attention over the last years. Anthocyanins, which are the most abundant flavonoid constituents of fruits and vegetables [7] might be of special interest in this respect. Recent data show that these compounds are excellent intracellular antioxidants even at very low concentrations [8] and it is their antioxidant activity that confers protection against carcinogenesis. However, in cells that have already undergone malignant transformation, anthocyanins, act as prooxidants and induce apoptotic death [9]. Various studies confirmed the antiproliferative and anticarcinogenic activities of anthocyanins in different cell lines originating from colorectal cancer [10, 11]. Colorectal cancer is the second most frequent cause of cancer-related death. A combination of various risk factors, including obesity, sedentary lifestyle, increased prevalence of smoking, excessive alcohol consumption, a diet rich in red and processed meat and low intake of fruits and vegetables, can trigger the carcinogenic process [12]. Anthocyanins have been shown to exert strong cytotoxicity on highly tumorigenic and metastatic Lovo/ADR colon cancer cell line while being less harmful to cells with low proliferation rate (Caco-2), which brings up their selective action [9]. Animal studies have also reported anticarcinogenic properties of anthocyanins. In induced rat colon cancer cell models they significantly decreased total tumors as well as aberrant crypts [13, 14]. Anthocyanins have also shown anti-inflammatory activity through targeting NF- $\kappa$ B pathway and COX-2 [15], which might have implications in tumorigenesis [16]. Even more, their ability to inhibit matrix metalloproteinase activity [17] could additionally contribute to both their anticancer and anti-inflammatory action. These activities, together with anthocyanins antioxidant properties, might also suggest their beneficial role in inflammatory conditions of the small intestine and colon, such as celiac disease and inflammatory bowel disease (IBD), yet there are no studies confirming this hypothesis.

Celiac disease is an immune-mediated disorder that affects primarily the small intestinal mucosa. It is characterized by chronic inflammation which occurs when gluten-activated Th1 cells start secreting cytokines (INF- $\gamma$  and TNF- $\alpha$ ) which in turn activate the release of enzymes such as metalloproteinases that can damage the intestinal mucosa leading to atrophy of intestinal villi. The progressive destruction of the small intestinal mucosa causes malabsorption, and a variety of clinical manifestations. Ulcerative colitis (UC) and Crohn's disease (CD) are the two main forms of inflammatory bowel disease. They are multifactorial conditions, where both genetic and environmental components play their parts. However, at the basis of UC and CD lays aberrant immune response and consequent inflammation regulated mainly by NF- $\kappa$ B. Both CD and UC are associated with a 5-fold increased risk of developing colon cancer [18].

## 2 AIMS IN TRANS2CARE PROJECT

Concerning the importance of diet on human health and the still scarce knowledge of the role of anthocyanins and bilitranslocase in both physiological and pathological conditions of the gastrointestinal tract, we will try to:

- A) Set dietary guidelines (based on the molecular mechanisms underlying anthocyanins bioavailability: their membrane transport at the level of the epithelial barrier of the gastrointestinal mucosa, and the role of BTL in it) for the prevention of chronic disorders of the intestine ;
- B) Study and exploit the drug transport potential of BTL in order to develop new orally-deliverable drugs on the basis of bilitranslocase substrate specificity;
- C) Exploit BTL as a biomarker: it might be an indicator of cell health (intactness), whereas its expression might be lost in cancer. It would be interesting to see how early the loss of expression occurs in disease development.

To reach these aims we will work in collaboration with IRCCS Burlo Garofalo (PP9), taking advantage of their tissue bank and expertise in gut histopathology in order to study BTL expression and determine any changes depending on the state of mucosa; with University of Nova Gorica (PP3), who has at its disposal sophisticated techniques for anthocyanins separation and analysis in order to measure their uptake and transport across different cell monolayers; and with ZTM (PP10), who can help us to prepare a monoclonal antibody against BTL for immunohistochemistry procedures.

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### **CONTACT INFO**

Jovana Čvorović and Federica Tramer are with the Department of Life Sciences, University of Trieste, Via L. Giorgieri 1, 34127 Trieste, Italy.