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Citation for final published version:

McKenna, Matthew, Linganathan, Saranya, Li, Amber, Ruge, Fiona, Lane, Jane , Ye, Lin , Jiang, Wen and Hargest, Rachel 2025. Human equilibrative nucleoside transporter 1 and concentrative nucleoside transporter 1 in colorectal cancer: What do we know? A systematic review. Histology and Histopathology 40 10.14670/HH-18-881

Publishers page: https://doi.org/10.14670/HH-18-881

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Human Equilibrative Nucleoside Transporter 1 and Concentrative Nucleoside Transporter 1 in Colorectal Cancer: What do we know? A Systematic Review

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Keywords

Colorectal Cancer
Nucleoside Transporters
Chemoresistance
Human Equilibrative Nucleoside Transporter 1 (hENT1)
Human Concentrative Nucleoside Transporter 1 (hCNT1)

Abstract

Colorectal cancer (CRC) remains a major global health challenge despite advances in screening, diagnosis, and treatment. This systematic review examines the roles of Human Equilibrative Nucleoside Transporter 1 (hENT1) and Human Concentrative Nucleoside Transporter 1 (hCNT1) in CRC, focusing on their expression, regulation, and impact on chemotherapeutic efficacy, particularly with nucleoside analogues like 5-fluorouracil (5-FU). We conducted a comprehensive literature search following PRISMA guidelines, yielding 29 studies that met our inclusion criteria. The review reveals variable expression of hENT1 and hCNT1 in CRC tissues compared with normal tissues, with implications for treatment response and development of resistance.

Increased hENT1 expression is associated with poor outcomes and resistance to 5-FU, suggesting its potential as a biomarker for predicting treatment response. Conversely, hCNT1's role appears more complex, with its expression influencing the efficacy of other chemotherapeutic agents like gemcitabine and capecitabine. The review also highlights the lack of robust, standardised methods for assessing mRNA and protein levels, which complicates the interpretation of data and the establishment of these transporters as reliable clinical markers.

Key findings include the potential therapeutic benefits of modulating hENT1 and hCNT1 expression to enhance drug efficacy and overcome resistance. The study underscores the need for further research using standardised and advanced methodologies, such as 3D cell culture assays, to better understand the mechanistic pathways and clinical implications of nucleoside transporter expression in CRC. Future research should aim to clarify the roles of hENT1 and hCNT1 in CRC and chemoresistance to develop targeted therapies and improve patient outcomes.

Abbreviations

- CRC - Colorectal Cancer
- hENT1 - Human Equilibrative Nucleoside Transporter 1
- hCNT1 - Human Concentrative Nucleoside Transporter 1
- 5-FU - 5-Fluorouracil
- mCRC - Metastatic Colorectal Cancer
- PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-Analyses
- MeSH - Medical Subject Headings
- ROC - Receiver Operating Characteristic
- TFT - Trifluridine
- TPI - Tipiracil Hydrochloride
- Tas-102 - Combination of Trifluridine and Tipiracil Hydrochloride
- PROSPERO - International Prospective Register of Systematic Reviews
- FTD - Tegafur
- 5'-DFUR - 5'-Deoxy-5-fluorouridine
- SLC28A1 - Solute Carrier Family 28 Member 1 (Gene encoding hCNT1)
- SLC29A1 - Solute Carrier Family 29 Member 1 (Gene encoding hENT1)
- Oncomir - Oncogenic MicroRNA
- NBMPR - Nitrobenzylthioinosine, an inhibitor of hENT1

Introduction

Colorectal cancer (CRC) continues to place a significant burden on the global population. Despite established and robust screening guidelines, increased efforts in public education, and advances in diagnosis and treatment, it remains the second leading cause of cancer deaths worldwide (Baidoun et al., 2020; Siegel et al., 2020). Recent epidemiological studies have revealed that the occurrence of CRC in those below the screening age is increasing year after year, with the greatest increase in diagnosis and associated deaths observed in those under 50 years (Siegel et al., 2020; Patel SG, 2022). This trend has been attributed to increased exposure to modifiable risk factors such as obesity, type II diabetes and physical inactivity, as well as genetic predispositions (Constantinou and Constantinou, 2024).

Whilst efforts in prevention are key, there continues to be an unmet need for effective CRC therapies, since those staged higher than stage I will typically require adjuvant therapy through chemotherapy and/or radiotherapy. As most screen-detected cancers present at stage I-II, and the majority of those with non-screen-detected CRC present at stage III-IV (Cardoso et al., 2022), the requirement for effective adjuvant therapies is self-evident.

Among the chemotherapeutic regimens utilised, the nucleoside-analogue 5-Fluorouracil (5-FU) stands as a fundamental treatment modality for CRC. However, the treatment response is as low as 50% in advanced disease (Dallas et al., 2009; Phua et al., 2013a) and nearly all those treated develop treatment resistance (Dallas et al., 2009). Mechanisms of chemoresistance or non-response observed in 5-FU and other nucleoside-based treatments involve cellular alterations that enhance cell survival, such as reduced drug uptake (Hruba et al., 2023a). The nucleoside transporter channels Human Equilibrative Nucleoside Transporter 1 (hENT1) and Human Concentrative Nucleoside Transporter 1 (hCNT1) are key to the intracellular uptake of purine and pyrimidine nucleoside chemotherapeutic drugs, used in the treatment of CRC and other highly differentiated cells (Pastor-Anglada et al., 2007; Pérez-Torras et al., 2013; Young, 2016). hENT1 is key in the transport of 5-FU, gemcitabine, and capecitabine, while hCNT1 has shown a high affinity for gemcitabine (Damaraju et al., 2003; Pastor-Anglada and Pérez-Torras, 2018; Puris, Fricker and Gynther, 2023). These channels have shown promise as both prognostic markers and potential therapeutic targets in haematological, pancreatic, gynaecological, breast and gastric cancers, yet, to date, there is relatively little research into their role in the development and treatment of colorectal cancer (Farré et al., 2004; Gloeckner-Hofmann et al., 2006; Ghazaly et al., 2015; Caparello et al., 2016; Shimakata et al., 2016; Hruba et al., 2023b; Puris, Fricker and Gynther, 2023). The systematic review aimed to study the evidence for the role of hENT1 and hCNT1 in the treatment of CRC and to highlight their potential in the development of chemoresistance in nucleoside-based treatments.

Materials and Methods

A systematic review of extant literature was performed in compliance with the PRISMA guidelines (Page et al., 2021), the focus being on original research on hENT1 and/or hCNT1 in colorectal cancer. The systematic review was registered and made available with PROSPERO [CRD42023462722]. PubMed/MEDLINE®, Embase™, and Web of Science were interrogated, covering all available records up to the search date (19-10-2023). The search used the exploded medical subject heading (MeSH) 'Colorectal Neoplasms' with relevant Boolean operators. The domain included the terms 'Adenomatous Polyposis Coli', 'Colonic Neoplasms', 'Colitis-Associated Neoplasms', 'Sigmoid Neoplasms', 'Colorectal Neoplasms, Hereditary Nonpolyposis', and 'Rectal Neoplasms' (Appendix 1).

The literature search and initial screening were performed using the publicly available Rayyan systematic review collaborative tool (Ouzzani et al., 2016), which enabled the blinding of two independent authors during the evaluation of titles and abstracts to exclude unrelated or duplicate studies. The remaining papers were retrieved for complete text review. Cross-referencing was implemented in studies meeting the inclusion specifications, maintaining the integrity of the blind review process. No 'Conflicts' between reviewers occurred following the complete text review; a third reviewer was identified in case any conflicts arose.

Inclusion and Exclusion Criteria

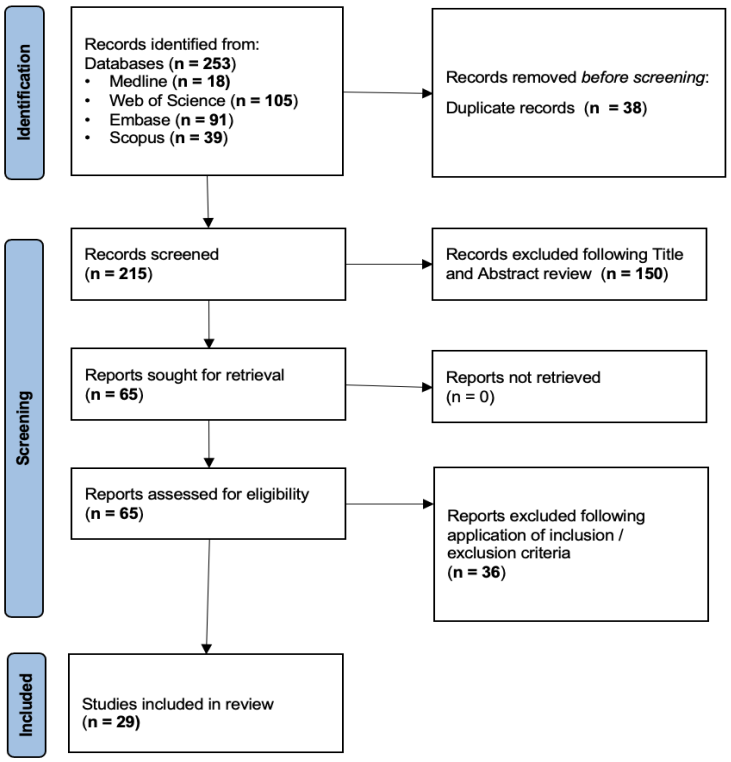
The review incorporated original research articles involving hENT1 and/or hCNT1 nucleoside-transport channels in colorectal cancer. Studies included experimental and observational research, genetic/genomic analyses, cell cultures, pharmacology, biochemistry, molecular modelling, and comparative studies related to colorectal cancer and hENT1/hCNT1 transporters. We focused on studies investigating disease progression, chemotherapy responses, transporter expression and functionality, cancer site, and patient survival rates.
Excluded were meta-analyses, editorials, opinion pieces, case reports, studies with insufficient transporter data, research on non-colorectal cancers, and duplicative datasets. Studies lacking relevant outcome data were also omitted, as were those not available in English.

Data extraction, outcome measures, and analysis

Common endpoints between papers were compared when statistically significant results were reported, and it was possible to perform.

Results

Figure. 1: Flowchart of Results of Literature Review



The database searches identified 253 publications, of which 38 duplicates were removed. The resulting 215 publications were screened, and 150 were removed after Title and Abstract review. All 65 of the remaining reports were retrieved, and of these, 36 were excluded for the reasons given in the Methodology section. Twenty-nine reports were included in the study (Fig. 1).

Expression and Localisation of hENT1 in Colorectal Cancer Cells.
hENT1 expression is ubiquitous throughout the colon and rectum (Pennycooke et al., 2001; Kunicka et al., 2016; Snezhkina et al., 2016). Through quantitative analysis of colon (n=11) and rectum (n=7) samples, Pennycooke et al. identified up to an 11-fold variation in hENT1 expression in non-cancer tissue samples, with no clear link between expression levels and anatomical site (Pennycooke et al., 2001).

As summarised in Table 1, increased hENT1 expression has been observed in biopsied human tissue and CRC and metastatic CRC (mCRC) cell lines (Liu et al., 2017). Kunicka et al. reported an average 2.5-fold increase in hENT1 mRNA expression in CRC tissue when compared with matched normal tissue in 151 human samples. Similarly, a small-scale study by Phua in seven Chinese patients demonstrated elevated hENT1 expression in CRC tissue, similar to that of Caco2 cells (Phua et al., 2013b).

However, heterogeneity in hENT1 expression exists within CRC. Analysis of biopsied tumours from CRC patients showed a consistent hENT1 mRNA expression with over-expression (up to 4-fold) in 27% of samples (n=18) (Pennycooke et al., 2001). This variation has also been shown in commercially available CRC and mCRC lines by Liu *et al.* (Table 1). Despite these variations, the literature consistently shows that hENT1 mRNA is expressed – and frequently upregulated – in CRC (Table 1). However, interpretation of these findings remains challenging without clinical correlation or direct comparison to non-cancerous colorectal cells.

hENT1 is a membrane-bound transporter that primarily localises to the basolateral membrane of colorectal epithelial cells, indicating a crucial role in nucleoside uptake from systemic circulation (Koichi Takahashi et al., 2015; Ueda, Hosokawa, and Iwakawa, 2015; Senyavina et al., 2016;

Fernandes et al., 2021). Research by Senyavina et al. demonstrated the functional expression of hENT1 on both apical and basolateral membranes of Caco-2 cells, using hypoxanthine transport fluxes as a marker (Senyavina and Tonevitskaya, 2015a, 2015b).

Although evidence is limited, it suggests an increase in hENT1 transcript expression in CRC. However, further investigation is needed to determine if this increase correlates at the proteomic level. Direct quantification of mRNA levels against protein expression, function, and localisation in both normal and cancerous colorectal cells is essential.

Expression and Localisation of hCNT1 in Colorectal Cancer Cells.
Efforts to quantify hCNT1 expression in colorectal tissues have produced variable results (Table 2). Pennycooke et al. and Ueda et al. reported undetectable mRNA levels in matched normal and cancer colon tissue samples and HCT116 cells, respectively (Pennycooke et al., 2001; Ueda, Hosokawa, and Iwakawa, 2015).

In contrast, Boces-Pascual et al. identified consistent mRNA expression across 17 samples of matched normal and colorectal cancer cell lines, as well as in HT29 and Caco-2 cells. When analysing the hCNT1 protein in biopsied tissue, there was a clear decrease in CRC samples when compared with normal tissue, using immunofluorescence analysis.
Notably, CRC cells grown in spheroid cultures displayed enhanced hCNT1 mRNA and protein levels and substrate uptake compared with monolayer cultures, suggesting that assays mimicking the physiological features of polarised cells may provide more representative models for studying hCNT1 and nucleoside transporters in CRC (Boces-Pascual et al., 2021). These findings are corroborated by *ex vivo* studies showing detectable hCNT1 mRNA in mouse and human CRC cells (Graham et al., 2011; Phua et al., 2013b) (Table 2).
hCNT1 is primarily localised to the apical (luminal) membrane of colorectal cells, where it acts as a sodium-dependent concentrative transporter (K Takahashi et al., 2015; Senyavina et al., 2016; Suenaga et al., 2017; Boces-Pascual et al., 2021). The apical to basolateral transport of nucleoside-derived chemotherapeutic agents in these cells is dependent on hCNT1 (K Takahashi et al., 2015; Suenaga et al., 2017).

Currently, evidence is limited regarding changes in the transcript and proteomic expression profiles of hCNT1 in CRC. With the development of oral chemotherapeutic agents, further investigation could establish hCNT1 as a critical therapeutic target in CRC. However, more research is necessary to fully understand its role and alterations in CRC.

Table 1: hENT1 Expression in Colorectal Cancer Cells

Research Study	Sample Type	Sample Count	Biomarker	Detection Method	Control Group	Findings
Pennycooke	CRC biopsy	18	mRNA	RNA Dot-blot, PCR	Matched Paired Normal Tissue	↑ 4-fold
Brim	Human Tissue	27	Methylation Status	Methylation-specific PCR	-	Increased in amplification mutations
Kunicka	CRC Biopsy	151	RNA transcripts	Real-time PCR	Matched Paired Normal Tissue	↑ 2.5-fold
Phua	CRC Biopsy	7	mRNA	rPCR	-	↑ 40% in Non-responders
	CaCo2	-	mRNA	qPCR	-	↑
Kim	HCT8	-	mRNA [3H]FLT Uptake	rPCR Radio-imaging	-	hENT1 mRNA detected ↑
	HT29	-	mRNA [3H]FLT Uptake	rPCR Radio-imaging	-	hENT1 mRNA detected ↑
	SW620	-	mRNA [3H]FLT Uptake	rPCR Radio-imaging	-	hENT1 mRNA detected ↑
	DLD-1	-	mRNA [3H]FLT Uptake	rPCR Radio-imaging	-	hENT1 mRNA detected ↑
	RKO	-	mRNA [3H]FLT Uptake	rPCR Radio-imaging	-	hENT1 mRNA detected ↑
	COLO 205	-	mRNA [3H]FLT Uptake	rPCR Radio-imaging	-	hENT1 mRNA detected ↑
	HCT116	-	mRNA [3H]FLT Uptake	rPCR Radio-imaging	-	hENT1 mRNA detected ↑
	LOVO	-	mRNA [3H]FLT Uptake	rPCR Radio-imaging	-	hENT1 mRNA detected ↑
Liu	Caco2	-	mRNA	qPCR	-	↑ 'Highly expressed'
	Colo320	-	mRNA	qPCR	-	↑ 'Highly expressed'
	HCT1	-	mRNA	qPCR	-	↑ 'Highly expressed'
	RKO	-	mRNA	qPCR	-	↑ 'Highly expressed'
	SW48	-	mRNA	qPCR	-	↑ 'Highly expressed'
	Colo201	-	mRNA	qPCR	-	↑ 'Highly expressed'
	Colo205	-	mRNA	qPCR	-	↑ 'Highly expressed'
	SW480	-	mRNA	qPCR	-	'Low' Expression
	HT29	-	mRNA	qPCR	-	'Low' Expression
	HCT116	-	mRNA	qPCR	-	'Low' Expression
	DLD-1	-	mRNA	qPCR	-	'Low' Expression
	T84	-	mRNA	qPCR	-	'Low' Expression
	SW626	-	mRNA	qPCR	-	'Low' Expression
	SW620	-	mRNA	qPCR	-	'Low' Expression
	SNU-C1	-	mRNA	qPCR	-	'Low' Expression
	SK-CO-1	-	mRNA	qPCR	-	'Low' Expression
	LoVo	-	mRNA	qPCR	-	'Low' Expression
Senyavina (1)	CaCo2	-	mRNA	qPCR	-	Highly expressed
Ueda	HCT116	-	mRNA Protein	PCR Western Blotting	-	Highly expressed
Snezhika	Genomic Database	-	mRNA isoforms	TCGA	Matched Normal Tissue data	↑ (4.7-fold)

Table 2: hCNT1 expression in Colorectal Cancer

Research Study	Sample Type	Sample Count	Biomarker	Assay Method	Control Group	Findings
Pennycooke	Human tissue	11 colon 7 rectal	mRNA	qPCR	Matched paired normal colon tissue	Not Detectable
Ueda	HCT116 cells	-	mRNA, Protein	qPCR	-	Not Detectable
Boces-Pascual	Human Tissue	17	mRNA	qPCRC	Matched paired normal colon tissue	No significant difference
	Human Tissue	4	Protein	IHC	Independent normal colon tissue	↓ in Tumour cells
	HT29 cells: Monolayer	-	mRNA, protein	qPCR, IHC	shRNA	Detectable
	HT29 cells: Spheroidal Assay		mRNA, protein	qPCR, IHC	shRNA	↑(3-fold) than Monolayer
	Caco-2 cells	-	mRNA	qPCR	shRNA	↓ than control
Phua	Human Tissue	7	mRNA	rPCR	-	↓ in non-responders to 5-FU
Graham	Murine tissue: hENT1 K/O	-	mRNA	qPCR	Wild type	↑ in hENT1 K/O cells
Temmink	H630 cells	-	mRNA	qPCR	-	Detectable

hENT1 as a predictor of treatment response in CRC.

hENT1 is recognised as a high affinity transporter for 5-FU (Choi J.-S. and Berdis, 2012; Kunicka et al., 2016; Hruba et al., 2023b). However, Phua et al. documented an average increase of 40% in hENT1 expression in non-responders to 5-FU-based treatment in CRC patients (Phua et al., 2013b). Similarly, work by Yoshinare et al. showed an inverse correlation between hENT1 mRNA expression and response to 5-FU in advanced CRC xenografts from 88 patients (Yoshinare et al., 2003). Analysis of 151 patients with CRC by Kunicka et al. found that those exhibiting higher hENT1 mRNA expression profiles displayed a 49% elevated risk of disease recurrence following curative surgery. This recurrence risk increased 3.5-fold in individuals with high hENT1 expression profiles who had previously undergone 5-FU treatment (Kunicka et al., 2016). Furthermore, through Receiver Operating Characteristic (ROC) curve analysis, Yoshinare et al. showed that hENT1 mRNA could serve as a sensitive predictor of 5-FU response, with a cut-off value of 0.4 (Yoshinare et al., 2003).

The mechanism underlying the relationship between elevated hENT1 mRNA expression and diminished sensitivity to 5-FU remains undetermined. Analysis by Kim et al. of eight human CRC cell lines (Table 1) revealed a direct correlation between hENT1 mRNA expression and the number of binding sites for radiolabelled substrate [3H]FLT. Notably, the number of binding sites significantly increases following exposure to 5-FU, despite a strongly negative correlation between basal hENT1 activity and 5-FU sensitivity (IC50 correlation coefficient of -0.86) (Kim et al., 2017).
These results imply that, although hENT1 protein functionality may be amplified, the effectiveness of 5-FU in CRC cells is concurrently reduced. This apparent contradiction is corroborated by findings in

Caco-2 cells, where the 5-FU response was enhanced following exposure to the selective hENT2 inhibitor, NBMPR (Phua et al., 2013b).

Elevated hENT1 expression in CRC cells may contribute to decreased 5-FU sensitivity by facilitating greater drug efflux. hENT1's bidirectional transport capacity permits movement along concentration gradients in colorectal cells (Senyavina and Tonevitskaya, 2015b; Ueda, Hosokawa, and Iwakawa, 2015). Senyavina et al. identified hENT1 on both apical and basolateral membranes, allowing vectorial flux of substrates such as 5-FU (Senyavina et al., 2016), highlighting the potential for enhanced apical efflux as intracellular concentrations of 5-FU increase. However, further work is required to directly quantify hENT1 expression and localisation in normal and cancer cells to better understand the correlation with the poorer response to 5-FU-based treatments.

hENT1 may contribute to the development of resistance to chemotherapeutic agents. Trifluridine (TFT), a nucleoside analogue structurally similar to 5-FU, relies on hENT1 for activity. It is used in combination with tipiracil hydrochloride (TPI) as the oral chemotherapeutic agent Tas-102 in metastatic colorectal cancer (mCRC), for patients who are 'non-responders' or resistant to other chemotherapies (Temmink et al., 2010; Suenaga et al., 2017). Temmink et al. observed a 2 to 3-fold decrease in hENT1 mRNA following metronomic TFT exposure in H630 cells, associated with a 10-fold reduction in intracellular TFT accumulation compared with naïve cells (Temmink et al., 2010). This suggests an alternative mechanism of hENT1-mediated chemoresistance, and further research with other drugs, including 5-FU, may help to understand why initial responders go on to develop resistance.

hCNT1 as a predictor of treatment response in CRC

The utility of hCNT1 as a prognostic biomarker of CRC treatment efficacy is uncertain due to inconsistent expression patterns in colorectal cells (Table 2). Phua et al. associated diminished hCNT1 mRNA with a reduced response to 5-FU treatment, though this finding was likely confounded by increased hENT1 levels, given that 5-FU uptake appears hCNT1-independent in CRC (Temmink et al., 2010; Choi J.-S. and Berdis, 2012; Phua et al., 2013b).

hCNT1 actively transports chemotherapeutic agents, including gemcitabine, capecitabine, FTD (tegafur), and 5'-DFUR, and its apical localisation suggests a significant role in the luminal absorption of these drugs in CRC (Damaraju et al., 2003; Choi J.-S. and Berdis, 2012; Suenaga et al., 2017; Boces-Pascual et al., 2021). Research by Boces-Pascual indicated a reduction in hCNT1 protein expression in CRC cells, with unchanged mRNA levels, when compared with matched normal tissue. Furthermore, there was a direct correlation between hCNT1 protein expression and the efficacy of 5-FDUR and gemcitabine, which was reversed through the inhibition of the Oncomir, miR-106a (Boces-Pascual et al., 2021). These results point to a post-transcriptional mechanism regulating hCNT1 expression. Further investigation into the signalling pathways of hCNT1 in CRC could enhance our understanding of its potential as a predictive marker of treatment responsiveness.

hENT1 and hCNT1 expression in metastatic Colorectal Cancer Cells

hENT1 expression in mCRC cells exhibits a complex pattern. Through analysis of human mCRC cell models, Liu et al. showed that 25% exhibit ‘over-expression’ of hENT1 mRNA, although the degree of expression was less pronounced than in primary CRC cells. Specifically, the primary CRC cell model SW480 demonstrated markedly higher hENT1 mRNA levels compared with its metastatic lymph node derivative, SW620 (Liu et al., 2017).

To date, there is a paucity of investigation specifically dedicated to exploring the expression of hCNT1 in mCRC, making this a pertinent area of research interest.

hENT1 and hCNT1 as co-expressed proteins in colorectal cells

The regulatory dynamics of nucleoside transport in colorectal cells involving hENT1 and hCNT1 co-expression are complex. Analysis of various human tissue datasets by Cesar-Razquin supports distinct gene expression patterns with SLC28A1 and SLC29A1, corresponding to hCNT1 and hENT1 not typically being co-expressed (César-Razquin et al., 2015). However, research in murine models indicated a predominant expression of mENT1 over mCNT1 in colon cells, with a compensatory increase in mCNT1 expression observed following mENT1 knockout, indicating a concerted function for effective nucleoside transport (Graham et al., 2011). These collective findings underscore the intricate regulatory mechanisms governing hENT1 and hCNT1 co-expression, which may have

implications for nucleoside transport and therapy in colorectal cells. Further exploration of their coordinated roles is warranted to clarify the precise nature of their interaction in CRC.

Discussion

As regards the findings of this systematic review, it is important to acknowledge its limitations. The body of research available is limited both in scope and scale, with studies often characterised by small sample sizes with limited clinical correlation. The heterogeneity in assay methodologies presents a further complication, leading to variability in results and interpretations across studies. Moreover, the absence of a collaborative standard for the measurement of mRNA and protein hinders the ability to draw robust, generalisable conclusions and further the understanding of the roles of hENT1 and hCTN1 in CRC.

Emerging evidence from this review suggests a consistent increase in hENT1 mRNA expression in CRC, which generally correlates with poorer outcomes and resistance to 5-FU treatment. Elevated baseline hENT1 levels are associated with an increased likelihood of non-response to 5-FU, as demonstrated by Phua's analysis of biopsied tissue and further supported by findings from Kim et al. and Yoshinare et al., who reported an inverse relationship between basal hENT1 expression and 5-FU efficacy in CRC cells *in-vitro* and *ex-vivo* (Yoshinare et al., 2003; Phua et al., 2013b; Kim et al., 2017).

These observations suggest that hENT1 expression could be a potential biomarker for predicting 5-FU treatment response in CRC, although the variability in expression observed among individual cases highlights the need for more comprehensive studies to fully determine the true positive predictive value of hENT1.

The mechanism behind the poorer prognosis linked to high hENT1 levels remains undetermined, yet evidence suggests that elevated expression may decrease drug sensitivity through the facilitation of drug efflux—a recognised factor in the development of drug resistance (Du et al., 2018; Azwar et al., 2021). hENT1 is expressed on both the basolateral and apical membranes of CRC cells, facilitating bi-directional substrate transport that could lead to enhanced efflux of nucleoside-based drugs. Although a direct correlation between mRNA and protein expression has not been fully established, the increased hENT1 activity noted by Kim et al. suggests a potential mechanism for reduced 5-FU cytotoxicity through augmented luminal efflux (Kim et al., 2017). This may explain the augmented response to 5-FU through selective hENT1 inhibition in CRC observed by Phua et al. (Phua et al., 2013b). Further research is required to investigate the proteomic changes in hENT1 within CRC cells and their influence on drug efflux and treatment outcomes. The recent identification of selective hENT1 modulators presents opportunities for developing novel therapies and enhancing the effectiveness of existing chemotherapy regimens in CRC (Rehan et al., 2019).

Resistance to 5-FU in CRC can develop after an initial response, possibly influenced by changes in hENT1 expression. Studies in pancreatic ductal adenocarcinoma have noted that hENT1 expression levels change from low to high during 5-FU-based therapy (e.g., FOLFIRINOX), correlating with depleted intracellular 5-FU reserves (46,47). Furthermore, in pancreatic cancer, increased hENT1 expression is associated with heightened sensitivity to gemcitabine, suggesting that modulation of hENT1 through 5-FU treatment could potentiate gemcitabine's effects (46–48). Investigating these therapy-induced alterations in hENT1 within CRC contexts could help to clarify resistance mechanisms in initially responsive patients and potentially refine therapeutic approaches (41).

Research on hCNT1 expression and its role in CRC has shown inconsistent results, likely due to its low endogenous expression. Boces-Pascual, however, demonstrated consistent hCNT1 mRNA and protein expression in biopsied tissues and CRC cell lines, noting a decrease in hCNT1 channel expression that correlated with reduced cytidine uptake and diminished efficacy of 5-FDUR and gemcitabine. The study also revealed that channel function and drug efficacy could be restored by silencing OncoMirs miR-17 and miR-106a. This research provides the first quantifiable evidence of hCNT1 expression, proteomics, and function in CRC, suggesting a role for hCNT1 in drug response and a potential mechanism of chemotherapeutic resistance via microRNA-mediated protein

downregulation. These findings align with observations in pancreatic cancer, where restoration of hCNT1 expression alone was sufficient to inhibit tumour growth, even without cytotoxic drugs (9).

hCNT1 may play a role in regulating nucleoside uptake in colorectal cells, with changes in expression influencing the rate of luminal uptake to maintain cellular nucleoside homeostasis. This is exhibited by its expression being related to hENT1 expression levels: increasing when hENT1 is knocked out and decreasing when hENT1 is overexpressed. These dynamics correlate with the previously published role of hCNT1 as a transceptor, beyond its primary role in nucleoside transport. This is further supported by its ubiquitous expression across various cell types, where it demonstrates functional redundancy (9).
hCNT1 exhibits functional adaptability in colorectal cells, with adjustments in its expression resulting in modulation of luminal nucleoside uptake, based on cellular nucleoside levels. Expression increases when hENT1 is knocked out and decreases with hENT1 overexpression, suggesting hCNT1's involvement in a feedback mechanism to maintain nucleoside homeostasis (26,34). Increased hCNT1 expression also enhances the efficacy of cytotoxic drugs (20), indicating its potential as a therapeutic target. Temmink et al. reported a 16-fold increase in hCNT1 levels in CRC cells following continuous exposure to TFT (41). Although this did not affect TFT uptake directly, this finding supports the potential of hCNT1 as a target in combination therapies with other hCNT1-transported drugs, like gemcitabine and capecitabine, which have shown improved antitumor effects in colon cancer xenografts, when used in combination (41,49). Further research into the signalling pathways regulating hCNT1 could enhance current therapeutic strategies and help to develop more effective treatments.

Given the low expression of hCNT1 in colorectal cells, particularly in cancerous cells, it has been proposed that 3D cell assays should be used to detect expression profiles more reliably (20). The application of these assays in cancer research is growing due to their closer resemblance to the *in-vivo* environment (47). However, these models are generally compared to 2D cell assays. The absence of direct comparisons to *ex-vivo* samples necessitates caution to ensure that the expression profiles observed in 3D assays accurately reflect the *in-vivo* state of HCNT1 in CRC.

Despite the growing body of research exploring the relationship between hENT1 and hCNT1 in CRC, progress in defining their roles in oncogenesis and their potential as therapeutic targets or treatment response markers has remained limited. This can be attributed, in part, to the utilisation of diverse cell models, variations in gene expression markers (mRNA versus protein levels), and the heterogeneity of sampled sources (commercial CRC cells, human and rodent tissues, and samples from individuals with prior 5-FU treatment). Nevertheless, current research findings strongly advocate for the establishment of a robust and reproducible model to comprehensively investigate these nucleoside transporters in CRC.

Given the luminal (apical) distribution of hCNT1 in colorectal cells, those with CRC who are deemed non-responders or resistant to 5-FU treatment may benefit from combination therapies with oral cytotoxic drugs. This, in combination with the aforementioned potentiation of 5-FU, noted with selective hENT1 inhibition, opens the possibility of enhancing both systemic and luminal uptake of cytotoxic drugs in CRC through the inhibition of hENT1. Further work should revolve around the effect inhibition of hENT1 has on hCNT1 expression and function. This could aid in the evaluation of hENT1 inhibition as a potential therapeutic target in the treatment of CRC.

Furthermore, the luminal expression of hCNT1 presents potential for targeted therapy in CRC cells exhibiting functional or elevated hCNT1 expression. Additionally, in the context of low rectal cancers, direct administration of hCNT1-mediated cytotoxic agents emerges as a potential avenue, offering more precise therapeutic interventions and potentially mitigating systemic side effects.

Conclusion

In conclusion, this systematic review underscores the complexities in defining the roles of hENT1 and hCNT1 in CRC. Despite limited and variable research findings, there is evidence developing that supports the involvement of these nucleoside transporters in the modulation of the drug efficacy of nucleoside-derived chemotherapeutics, particularly 5-fluorouracil and hENT1. The review highlights the dynamic expression profiles of hENT1 and hCNT1 in CRC and their potential as biomarkers of treatment response. However, significant challenges remain due to methodological differences across studies, such as assay heterogeneity and the scale of experimental models. Future research should

focus on standardised and reproducible approaches that are reflective of the *in-vivo* environment to enhance our understanding of these transporters in CRC treatment paradigms. Further investigation into the signalling pathways and the interplay between hENT1 and hCNT1 could lead to more effective and tailored therapeutic strategies.

Declarations of interest: None
Declaration of relevant funding: None
Conflicts of interest: None

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Appendices

Appendix 1: MeSH Tree Structure for 'Colonic Neoplasms'

- Neoplasms [C04]
  - Neoplasms by Site [C04.588]
    - Digestive System Neoplasms [C04.588.274]
      - Gastrointestinal Neoplasms [C04.588.274.476]
        - Intestinal Neoplasms [C04.588.274.476.411]
          - Colorectal Neoplasms [C04.588.274.476.411.307]
            - Adenomatous Polyposis Coli [C04.588.274.476.411.307.089]
            - **Colonic Neoplasms [C04.588.274.476.411.307.180]**
              - Colitis-Associated Neoplasms [C04.588.274.476.411.307.180.400]
              - Sigmoid Neoplasms [C04.588.274.476.411.307.180.800]
            - Colorectal Neoplasms, Hereditary Nonpolyposis [C04.588.274.476.411.307.190]
            - Rectal Neoplasms [C04.588.274.476.411.307.790]