

NON-CODING RNAs, AS BIOMARKER AND AS A THERAPEUTIC MODALITY FOR COLORECTAL CANCER

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ABSTRACT

Non-coding RNAs (ncRNAs) have been a matter of interest since time of their discovery in late 1950s. There are various types of ncRNAs available like the micro RNAs, long non-coding RNAs (lncRNAs), small nucleolar RNAs (snRNAs) and Piwi Interacting RNAs (piRNAs). They itself do not code for any proteins but plays a vital role in progression of many fatal diseases by their dysregulation. Similar case is also for colorectal cancer, a life-threatening disease that comes as a bane with mal-lifestyle practices. Various ncRNAs are often abundantly found in different colorectal cancer patient samples like the miR-17-92, miR25, H19, Xist, SNORD44, circRNA-000014, whose hyper production promotes colorectal cancer cell growth, metastasis and cancer cell survival. These biomolecules act as the biomarker for the disease as they are very particular with their implication in colorectal cancer. So makes an advance opportunity for faster diagnosis of the patients. Another novel approach to suppress colorectal cancer is via ncRNA mediated gene silencing. The target mRNAs like the miR25, MALAT-1, SNORD44 have oncogenic activity and are related to the advent of colorectal cancer in the patients which can be silenced by application of complementary therapeutic ncRNAs thereby progression

of the disease can be effectively controlled. Recent studies even focus on role of Probiotics as potential anti-CRC agents as these are strains of gut microbes whose active metabolites has been proven to suppress CRC progression with greater efficiency than many of the modern medications.

The review focuses on joint approach towards diagnosis and cure of colorectal cancer, an emerging world problem with the ncRNAs as diagnosis biomarker mediated by RNA-seq read mapping, real-time RT-PCR and eventual suppression of the dysregulated ncRNA via ncRNA mediated gene silencing and intake of potential Probiotics.

Keywords:Colorectal Cancer, Gene Silencing, Non-Coding RNAs, RNA Biomarkers,Probiotics

Introduction

Non-coding RNAs are the type of RNAs that do not code for proteins, yet play a significant role in regulating gene expression and other cellular functions. First Non-coding RNA was discovered by George Palade and Paul Zmecnik in mid 1950s. Their dysregulation has been implicated in various diseases, including cancer. These are often identified to be disease biomarkers for several carcinomas and often being used as diagnosis monitor to predict the progression of the disease in the patients. There are multiple variants of non-coding RNAs available such as the Micro RNAs, Long Non-coding RNAs, Small Nucleolar RNAs and Piwi Interacting RNAs (John S Mattick, Igor V Makunin., 2006). These have an impressive role to play as potential cancer therapeutics and Carcinoma Biomarkers (Yokugawa Y et al., 2015). Recent implications of circular non-coding RNAs in cancer therapy have been a revolutionary work as it kills the major drawback that was arising with the use of therapeutic linear non-coding RNAs as the former are covalently closed in nature and are resistant to cellular nuclease resulting in increase of their half-life. Dysregulation of non-coding RNA refers to the phenomenon of abnormal expression, processing or functioning of ncRNAs which leads to severe and chronic diseases and even loss of cell cycle, arrest of cellular check points leading to tumorous outgrowths and ultimately cancer. Over and under expression of ncRNAs causes mutations

leading to loss of vital functionality of the cell or even gain of new functionality leading to cellular abnormalities. This may even cause epigenetic modifications like changes in DNA methylation and Histone modification. Over the time ncRNAs have been under strict observation as their abnormalities can be a triggering point for any kind of diseases, and abnormal behavioural patterns of ncRNAs serves as the first ever alarming symptom for cancer as this starts happening at a very early period before the progression of the disease to its fatal nature, thus considering ncRNAs as biomarkers for life threatening diseases like cancers helps easy and early diagnosis of the patient so that proper treatment could be given to prevent further progression of the disease. Recent studies with the virtue of RNA-seq read mapping revealed that upregulation of four long non-coding RNAs namely CRCAL-1, CRCAL-2, CRCAL-3, CRCAL-4 have been seen in case of colorectal cancer and so the above colorectal cancer associated long non-coding RNAs can be used as perfect biomarkers for the disease (Atsushi Yamada et al., 2018).

Using ncRNAs as an effective molecular tool for gene silencing have been used since date of its discovery. This process effectively subdues the targeted gene by the mechanism of complementary hybridization, this means the ncRNAs are selected such that they are complementary to the target mRNA sequences to silence the effect of the corresponding gene, this binding of the ncRNA to the target mRNA is due to presence of complementary nitrogenous bases and referred as RNA hybridization. This acts as a novel anti-angiogenic targeted therapy. (A Mulkeen et al., 2004).

Recent research has revealed the fact that probiotics have strong impact in suppression of CRC even with better efficiency than any other modern medications. These are actually strains of human gut microbiome whose active metabolites cause cytotoxicity to CRC cell lines (Hold GL et al., 2016).

Application of the mentioned processes paved a life-saving path towards faster diagnosis and treatment of the colorectal cancer patients. Chronic nature of the disease that can make its way into the body due to certain mal practices like smoking,

unhealthy diet, obesity, alcohol consumption etc that comes together with the modern lifestyle. Curing of the disease becomes a vitality to suppress the adverse effect of the bad lifestyle choices and save millions of lives annually.

ncRNAs as biomarkers for colorectal cancer

Biomarkers are the molecular tools to make a birds view over the occurrence and progression of a disease. Studies revealed that there is pivotal relation with over expression of particular ncRNAs that comes with the advent of a particular disease as a result monitoring the abnormalities of these molecules could make out a gradual relation to the occurrence of the disease and can be used as a detection tool. Previously colonoscopy was the only reliable method for the detection of colorectal cancers but eventually this is avoided due to its high cost and longer time consumption. Hence need of biomarker discoveries for better and faster diagnosis of the disease with greater proximity and yet cost cutting. Proteins are being used these years as biomarkers for colorectal cancers.

M2PK, MMP9 and RBP4, TFF3 are the important protein biomarkers that have been found profusely in the patient stool and blood serum samples respectively(Alexandre Loktionov et al.,2019). Another such biomolecule that has high potential to be a biomarker for colorectal cancer are the ncRNAs. There have been extensive research showing that over expression of certain ncRNAs are associated with the advent of the colorectal cancer. The case of their over expression is suspected to be the hypoxia condition that is inducing the cell to become cancerous (Hsiao-Mei Chao et al.,2022).Some of the pseudogene that are produced by some ncRNA that epigenetically silences tumour suppressors one such example is a pseudogene derived from lncRNA DUXAP10 that promotes colorectal cancer growth by suppression of p21 and pTEN(Yifan lian et al.,2017). Apart from siRNA, miRNA and LncRNA biomarkers there are also piRNAs.The piRNAs are produced from pre-piRNAs by a dicer independent process, such piRNA whose up regulation is seen in case of CRC are piR-1245 and piR-823(Mandana AmeliMojarad et al.,2022). Some of the vital ncRNAs that have shown over expression in the colorectal cancer patients have been listed below in Table

1 along with their sample sources and types of those ncRNAs are mentioned.

Table 1: List of vital ncRNA, their biomarkers with the source

Slno.	Biomarker	Type of ncRNA	Source sample	Reference
1	CRCAL-1,2,3,4	lncRNA	Colorectal neoplasms	Atsushi Yamada et al.,2018.
2	CRNDE	lncRNA	CH16 of CRC	Jie Ding, Juan Li et al.,2017
3	CASC9	lncRNA	HT-29 and SW680 cell line	Hui-Zi Li et al.,2021
4	BLACAT1	lncRNA	Blood serum	Alexandre Loktionov et al.,2021
5	MiR-92a	miRNA	CRC cells	Xiaoya Luo et al.,2011
6	piR-1245	Piwi-interacting RNA	CRC cells	Mandana AmeliMojaradet al.,2022
7	Circ-HIPK3	circRNA	Urine	Mandana AmeliMojaradet al.,2021

Detection of these ncRNAs in high amount in the mentioned sample of the suspected colorectal cancer patient are to be done via RNA-seq read mapping and their dysregulation can be further validated using real-time RT-PCR(Atsushi Yamada et al.,2018). This process of patient diagnosis has been found cost effective and less time consuming which paved out crucial opportunity for the patient to proceed with the treatment.

mRNA silencing approach to suppress Colorectal Cancer

In the contemporary period, gene silencing has evolved to be a novel tool for suppression of deadly diseases like the colorectal cancer. Any type of therapeutic application needs a biological target at molecular level which is to be controlled. Non-coding mRNA mediated gene silencing is a process in which neutral non-reactive ncRNAs are produced that are complementary to the target such that when they are placed into the same environment with the target which is specifically an mRNA, binds to it by complementary base pairing and as a result the effect of the very specific gene whose transcribed product is the targeted mRNA, is suppressed. These ncRNAs are produced by the action of an enzyme called dicer from double stranded RNA and get loaded over their target by the virtue of RISC complex, that means RISC helps in location and proximation of the target mRNA (Gregory J Hannon.,2002).

Studies successfully demonstrated the therapeutic anti-colorectal cancer activity of the ncRNAs as in case of a lncRNA derived pseudogene DUXAP10 that was initially used as diagnosis biomarker from HCT116 and SW480 cell lines, but later studies revealed its biological role in colorectal Cancer cell proliferation and metastasis as it is associated with knocking down of p21 and pTEN tumour suppressors. Knocking down this lncRNA DUXAP10 using DUXAP10 siRNA led to higher apoptotic levels in the CRC cells in comparison to the control cell line which was not transfected with the mentioned siRNA (Yifan Lian et al.,2017). Another lncRNA upregulation was noticed in case of CRC that is lncRNA CASC9. On silencing this lncRNA CASC9 in HT-29 and SW680 cell lines using si-CASC-1 or si-CASC-2, CRC cell proliferation and growth was hampered. This was because on silencing CASC9 depletion in cyclin D1 and CDK2 was noticed that are associated to G1 cell cycle arrest (Hui-Zi Liu et al.,2021). Another Long non-Coding RNA whose deregulation causes CRC is CRNDE that is located on human chromosome 16. Knockdown of this lncRNA using si-CRNDE 1 and si-CRNDE 2 increased apoptotic levels in CRC cells. It was also experimentally proven that down regulation of lncRNA linc-00152 by miR-376c-3p miRNA restricts viability

of CRC cells(Jie Ding , Juan Li et al.,2017).Circular ncRNA are one of their kind, discovered in1990s.The are synthesized without intervention of dicer enzymes, by following ways : Exon reverse splicing into loops(exonic circRNA,ecircRNA), intron-preserving transcript reverse splicing (exon-intron circRNA, elcircRNA), and intron reverse complementary pairing (circular intronic RNA, ciRNS). Two of the circRNA namely hsa_circ_0066631 and hsa_circ_0082096 are seen to be up regulated in CRC stem cells and targets and downregulates CRC stemness miRs such as miR-382, miR-548c-3p and miR-579(Xiangyu Jian et al.,2020). NUSAP1(aberrant nucleolar and spindle associated protein 1) is a highly conserved protein in humans and recently its upregulation has been specifically noticed in case of CRC cell lines such as CACO2, LoVo, LS274T and SW480. On silencing the mRNA responsible for NUSAP1 production by NUSAP1 siRNA, adequate lowering in cell growth and proliferation of CRC cells is noticed (Guoda Han et al.,2019). A lncRNA PANDAR that was previously suspected only as a biomarker for CRC, have shown a peculiar phenomenon that when silenced jointly by PANDAR siRNA 1,2 and 3 increased apoptotic rates in CRC cells even at relatively low doses of Curcumin, a drug use to treat CRC. This serves as a joint therapy in combating CRC.

Combined Therapy for Combating CRC: ncRNAs and Probiotics

The review has enlisted the role of ncRNA in CRC therapy both as a diagnosis biomarker and as a molecular tool to silence dysregulated villain molecules and thus are proven to be a boon for controlling CRC cell proliferation and metastasis. This have been proven to be much cost cutting yet latest and more the most effective tool to replace orthodox methods of cancer diagnosis and treatment such as colonoscopy and chemotherapy. Studies even revealed that probiotics plays a significant role to supress CRC cell proliferation as these are always naturally directed into the gut. Probiotics are practically strain of gut microbes that comes along with the food we intake and exerts a positive impact to our health. The positive role of probiotics has been confirmed as they demonstrated that active or inactive strain

of probiotic like *Lactobacillus acidophilus* and *Lactobacillus casei* have high apoptotic capacity over 5-fluorouracil (5-FU) of CRC cell line LS513 (Baldwin et al.,2010). Not only the live or inactive bacteria but also the cell supernatant of probiotic strains *L.casei* and *L.rhamnosus* can successfully suppress CRC progression. CFS of above the mentioned strains were proven to significantly reduce HCT-116 cell invasion. Inhibitory activities were not found when HCT-116 cells were exposed to commensal bacteria *Bacteroides thetaiotaomicron* CFS. The active molecules were detected in the 50-100kDa and >100kDa fractions of *Lactobacillus* strains' CFS. The research established that *L. casei* and *L. rhamnosus* GG secretory metabolites have anti-invasive effects on HCT-116 cells (Escamilla J et al.,2012). Further research identified that *L. johnsonii* BCRC17010 was an effective probiotic strain with high adhesion capacity and proapoptotic process induction and lactate dehydrogenase release in HT-29 cells and efficiently suppressed the growth of HT-29 cells (Chen et al.,2017). Methanolic extract of secreted metabolites of *Pichia kudriavzevii* AS-12 (MEPK) was found to be cytotoxic to Caco-2 and HT-29 cells and the cytotoxicity in HT-29 cells was found to be equivalent to that of 5-FU. BAD, caspase-3, caspase-8, caspase-9, and Fas-R expression increased, whereas the expression of Bcl-2 was inhibited in MEPK-treated cancer cells. The expression level of proapoptotic genes (caspase-3, caspase-9, Fas-R in HT-29 cells, and Fas-R in Caco-2 cells) was greater in MEPK treated cells compared to 5-FU treated cells (positive control). According to the findings, the MEPK can be regarded as an effective anti-colorectal cancer agent (Saber et al.,2017).

Conclusion

Colorectal Cancer is the third largest cancer in the world leading to millions of deaths every year. Classical treatment of colorectal cancer like other cancers are very unpredictable and tedious with colonoscopy, chemotherapy and other modern medication. Advent of RNA therapy to combat CRC paved a way towards faster diagnosis and better suppression of CRC in the patients. This can be also conducted on severe Chemo-resistant patients creating another chance for their

survival. ncRNA biomarkers have been identified to be the most valid and efficient tool for cancer diagnosis. Targeting the ncRNA biomarker have increased the efficiency of the ncRNA mediated gene silencing therapy in CRC cells. Apart from these probiotics have been also proven to be a magic supplement in controlling cancer cell proliferation. These foods are often easily available and are not majorly subjected to cause any allergic reactions in the patients. The active compounds of probiotics even showed better anti-cancer effect that many modern medicines. This review linked up all the possible ways to combat CRC sustainably in a less cost-effective way expecting to promote awareness and to make cancer treatment within the means of common folk. Diagnosis using ncRNA biomarkers and cancer control using ncRNA mediated gene silencing and Probiotics have potential to be the most effective combined therapy to CRC saving millions of lives globally.

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