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Review Article

Critical Role of Sestrin2 in Regulating Nrf2/Keap1 Pathway and Mediating Autophagy in Colon Cancer

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Abstract

Colon cancer also called colorectal cancer has become one of the deadliest cancers with incidence rates of 4.3% and 4.10% in men and women respectively. It has been reported that CRC is mainly due to cellular/ER stresses such as hypoxia, which may lead to the accumulation of reactive oxygen species (ROS). So, it is essential to scavenge these ROS. Sestrin2 (Sesn2) is known to suppress ROS accumulation by regulating various signalling pathways such as Nrf2/Keap1 pathways and maintaining mitochondrial homeostasis through the AMPK/mTOR pathway etc., thereby inducing autophagy. This review provides an overview of the Nrf2-Keap1 signalling pathway, the dual role of Nrf2, and autophagy in cancer, along with the role of sestrin2 in triggering autophagy in colon cancer cells.

Keywords: Colon Cancer; Cellular/Er Stress; ROS; Homeostasis; Sestrin2; Nrf2/Keap1 Signalling Pathway; Autophagy

Introduction

Nrf2 is a transcription factor that regulates the expression of antioxidant proteins and is an emerging regulator of cellular resistance to oxidants. In several cancers, Nrf2 plays a multifunctional role in association with Kelch-like ECH-associated protein1 (Keap1) in its signalling pathway which induces pro-survival genes that may promote cancer cell proliferation by metabolic reprogramming. On the other hand, Nrf2 is regarded as a tumor inhibitor that controls oxidative stress and has an essential role in [1].

It has been reported that Sestrin2, also known as hypoxia-inducible gene-95 (Hi95) which is encoded by the Sesn2 gene is known to exhibit anti-cancer activity in CRC, conferring tumor suppressor activity thereby inhibiting colon carcinogenesis. The main mechanism through which Sesn2 exhibits its activity is by mediating [2]. Macroautophagy is a self-digesting mechanism that involves several conserved autophagy - related proteins (Atg). It is stimulated under conditions of cellular stress that is mainly due to the accumulation of ROS which leads to an increase in overall cellular oxidative stress levels, eventually, mitochondria-mediated cell death occurs. However, in cancer, autophagy plays a dual role like a double-edged sword that suppresses tumorigenesis by inhibiting cancer-cell survival and inducing cell death. On the other hand, it also facilitates tumorigenesis by promoting cancer cell proliferation and tumor growth [3]. Several mechanisms have been reported to induce autophagy in colon cancer. Among which autophagy induced by Nrf2/Keap1 signalling pathway, mediated by Sestrin2 is pivotal and will be discussed further.

ROS-induced ER stress in colon cancer

Accumulation of ROS and redox imbalance leading to ER stress is the main characteristic feature seen in most cancer cells. Imbalanced redox homeostasis and ROS play a crucial role in tumor progression and metastasis. ROS-mediated ER stress pathway induced by levistolide A has been reported in colon cancer cells [4]. Treatment of colon cancer cells with andrographolide resulted in increased levels of ROS, inducing ER oxidative stress and disruption of mitochondrial membrane potential. Andrographolide also

increased the levels of expression of antioxidant genes and ROS accumulation downregulates cell cycle and cell survival pathways, thereby inducing cell death [5]. Docosahexaenoic acid (DHA) treatment induced ER stress on SW480 and SW620 CRC cell lines, thence resulting in growth arrest and protein degradation [6]. HT29 cells treated with resveratrol induced significant ER stress markers and dose-dependent apoptotic cell death suggesting induction of ER stress was reported [7]. CT26 xenograft mouse model showing significant cell death when treated with curcumin derivative-WZ35 by a ROS-ER stress-mediated mechanism has been reported [8] Mitochondrial-associated cell death (mitophagy) was reported in human colon cancer cells (HCT116) through ER-stress-induced autophagy when treated with bis-Dehydroxy curcumin [9] Increased intracellular ROS levels and sestrin2 expression were reported in Quercetin-treated HCT116 human CRC cell lines [10].

Duality role of Nrf2 in cancer

Nrf2 is mainly involved in defending cells against metabolic, xenobiotics, and oxidative stress and is important for maintaining tissue integrity. It is a critical determinant in oncogenesis and is found in many types of cancer including colon cancer. Nrf2 is regarded as a tumor inhibitor; however, deregulated Nrf2 activity promotes carcinogenesis. Evidence shows that Nrf2 acts itself as a proto-oncogene or it supports the transforming potential of other oncogenes. Under normal conditions, Nrf2-dependent cytoprotective effects prevent cellular damage and dysfunction. Nrf2 deregulation confers growth advantages to cancer cells, which includes proliferation, anti-apoptosis, and resistance to drugs as well as radiotherapy.

Prolonged activation of Nrf2 favours progression in several types of cancer including colorectal cancer. Increased levels of Nrf2 in cancer cells promote proliferation by maintaining redox balance and generating antioxidant activity in cancer cells. Several studies showed that enhanced glutathione synthesis and elevated levels of glutamine for its synthesis support cancer cell proliferation. Increased activity of Nrf2 in cancer is due to several mechanisms that have been reported, among which deranged interaction between Nrf2 and keap1, modifications of keap1, and epigenetic silencing of keap1 were mainly seen [11].

Nrf2 as a tumor suppressor

Anti-tumorigenic effects of Nrf2 have been confirmed with the *in vivo* studies that showed Nrf2 gene ablated mice were more vulnerable to colonic inflammation and colon cancer formation when treated with colitis-inducing chaotropic agents such as dextran sulfate sodium and azoxymethane [1] In some cases of colon cancer, expression of Nrf2 reduces cell proliferation and increases apoptosis of cancer cells were also reported. Another study found that Nrf2 expression promoted indirectly by repressing or silencing keap1 reduced the risk of CRC [12]. Knockdown of Nrf2 induces growth inhibition, promotes apoptosis, and inhibition of migration was reported in SW480 cell lines of CRC [13].

Nrf2 as a tumor promoter

Even though Nrf2 has invaluable vital role in cytoprotection its activation for a prolonged period makes it a pro-tumorigenic factor. Nrf2 spiking ranges from a frequency around 81-95% in colorectal cancer. Several studies showed that about 20% of CRCs are due to disruption of keap1 expression, showing decreased levels of keap1 and enhanced Nrf2 activation, which makes it pro-tumorigenic [2]. In colon cancer cells, overexpression of Nrf2 is related to tumor progression and poor prognosis. Nrf2 increases CRC risk by promoting angiogenesis and uncontrolled proliferation [14]. Overexpression of Nrf2 induced by chemical agents such as T-BHQ resulted in increased O2 consumption leading to higher VEGF and HIF-α signalling, eventually increased angiogenesis and tumor growth were reported in colon cancer [15].

Nrf2/Keap1 signalling pathway in colon cancer

Keap1 is a negative regulator of Nrf2 which is bound to Nrf2 under normal conditions. Nrf2 translocate to the nucleus in response to oxidative stress, attaches to the antioxidant response element (ARE), activates downstream genes, and performs physiological functions. As a result, Sesn2's antioxidant action is mediated via p62-dependent keap1 autophagy activating Nrf2. Thus Keap1 serves as an oxidative stress sensor [2]. Upregulation of Nrf2 gene expression shows elevated levels of Nrf2 protein in response to ARE-inducers. A study proved that nitric oxide treatment activates Nrf2/Keap1 signalling pathway in HCT116 (human colon cancer cell lines) cell lines by triggering Nrf2 dissociation from [16]. Evidence proved that Nrf2 regulates more than 200 genes that are involved in cellular processes and cytoprotection including genes that encode for autophagy such as ATG5, ATG7, and LC3B. The pathway plays a chief role in the metabolic reprogramming of cancer cells that induces cancer cell proliferation and progression. Activation of PI3K/AKT signalling pathway increases nuclear translocation of

Nrf2, independent of keap1, allowing Nrf2 to promote metabolic reprogramming and increase cell proliferation [17]. Nrf2 is rapidly degraded by proteasomes when keap1 forms ubiquitin E3 ligase complex with CUL3 and Nrf2. Thus, under the absence of cellular stresses, Nrf2 is constantly degraded by keap1. But, when keap1's cysteine residues were modified or deformed under conditions of cellular stress, the activity of ligase is diminished, thereby activation of Nrf2 occurs. Activation of the Nrf2/keap1 signalling pathway provides antioxidant effects in normal cells but induces apoptosis in cancer cells [18]. Prolonged activation of Nrf2 in tumor progression is maybe due to genetic alterations that affect Nrf2/Keap1 pathway or from long-term exposure of epithelial cells to constant oxidative stress. Studies show that precursor lesions of tumors exhibit high Nrf2 activities with considerable prevalence where Nrf2 exerts pro- and anti-tumorigenic activities. Under these conditions, Nrf2 is directed to act as an oncogene [1]. Nrf2/HO-1 axis has been shown to play a vital role in colon cancer survival and aggressiveness. Hemo-oxygenase-1 (HO-1) is an ARE-regulated phase II detoxifying enzyme regulated by Nrf2. A study demonstrated that activation of ER marker-CHOP (C/EBP-homologous protein) promotes autophagy in colon cancer cells (HCT116). Ethanol (EtOH) treatment stimulated a pro-survival effect by inducing autophagic flux in HCT116 cells. Upon treatment with ethanol, nuclear translocation of both Nrf2 and HO-1 and activation of Nrf2-dependent anti-oxidant pathway with enhanced levels of Nrf2 were reported in their western blot analysis. Furthermore, both Nrf2 and HO-1 activation has been shown to increase tumor growth by increasing pro-invasive and angiogenic factors such as VEGF and MMPs [14].

Role of SESN2 in colon cancer

Studies suggest that Sesn2 is an important regulator of mTOR activity that inhibits colon carcinogenesis. *In vitro* analysis proved that 5-fluorouracil (5-FU) induces Sesn2 expression and inhibits invasive behaviour of colon cancer cells through upregulation of Sesn2 in colon cancer cell lines (HCT116 and HT29). 5-FU induces expression of Sesn2 in a p53 dependent manner. Sesn2, in turn, suppresses ROS accumulation and negatively regulates the mTOR signalling pathway. Knockdown of Sesn2 reversed 5-FU mediated decrease in cell migration [19]. Docosahexaenoic acid (DHA) promoted oxaliplatin-induced cell viability reduction and autophagy induction via ER stress induction and Sesn2 activation. Sesn2 plays a vital role in autophagy and in colon carcinogenesis and knockdown of which partially decreased oxaliplatin- and DHA-induced autophagy. This study proved that oxaliplatin and DHA in combination induced autophagy in CRC cells (HCT116), mediated by overproduction of ER stress which leads to increased sestrin2 activity [20]. Evidence suggests that Sesn2 confer tumor suppressor activity since they suppress TORC1 activation. Decreased expression of Sesn2 leads to an unfavourable prognosis in CRC. Sesn2 inhibits colon carcinogenesis by negative regulation of the mTOR signalling pathway has been reported [21].

Sesn2 and P53 in human perspective

Human Sesn2 expression is elevated in colitis but it is lost upon downregulation of p53 during colon carcinogenesis. An analysis showed that in ulcerative colitis, levels of sestrin2 were elevated, whereas, in colon cancer, a very low level of sestrin2 was seen. *In vivo* mice studies showed that colon cancer associated with inflammation grew faster in mice that lacked the gene for sestrin. Sesn2 is crucial for the appropriate regulation of mTORC1 and ER stress pathways during colon mucosae damage and thereby it functions as an antagonist of colitis and colon cancer development. Murine model studies showed that Sesn2 loss selectively leads to mTORC1 hyper activation and promotes colitis-induced colon cancer growth [22] *In vitro* and *In vivo* analysis reported that overexpression of Sesn2 decreases ROS production and suppresses cell proliferation through the AMPK/mTORC1 pathway. Elevated of Sesn2 was found to inhibit CRC [23].

Upregulation of Sesn2 inhibits proliferation and triggers apoptosis in CRC cell lines (SW620) by deterring mTORC1 and activating AMPK. The Significant role of Sesn2 in growth inhibition of CRC induced by quercetin and 5-FU suppressed proliferation and induced apoptosis in CRC cell lines HT29/HCT116 by targeting the Sesn2/AMPK/mTOR pathway has also been reported [24]. *In vitro* and *In vivo* studies attested that Heme (Fe) induced stress by Hemin on colon cancer cell lines (HCT116, RKO, MC38) triggered the expression of Sesn2 by activating ROS and Nrf2, thereby contributing more resistance to hemin-induced apoptosis in colon cancer cells and promoted tumor growth in MC38 cell line. Though Sesn2 is known to suppress tumorigenesis, it may also promote tumor growth under an iron-rich environment [25]. Immunofluorescence and western blot analysis proved that expression of Sesn2 was significantly lower in SW480, SW620, LoVo and HT29 human CRC cell lines when compared to a control human FHC (Fetal human cells-normal Fetal colonic mucosa). Clinical studies compel that in

almost all cancers Sesn2 expression is decreased and downregulated. Thence, lower levels of Sesn2 may augment oxidative stress, thereby provoking tumor metastasis [26].

Evidence suggesting autophagy mediated by Nrf2/Sesn2 in colon cancer

Macroautophagy or autophagy is a self-degradation process that is stimulated under conditions of cellular stress. Autophagy exhibits duality in cancer. It subdues tumorigenesis by deterring cancer cell survival and inducing cell death. Contrastingly it facilitates tumorigenesis by promoting cancer cell proliferation and tumor growth. It plays cytoprotective roles in maintaining cellular homeostasis and for the maintenance of genomic stability.

Accumulation of ROS elevates overall cellular oxidative stress levels, eventually, mitochondria-mediated cell death occurs. Despaired counter tumor response is closely associated with the accumulation of defective mitochondria, suggesting mitophagy impairment. Mitophagy promotes plasticity in cancer stem cells through metabolic reprogramming for better adaptation to the tumor microenvironment. Dysregulated mitophagy results in the accumulation of damaged mitochondria, which plays a role in carcinogenesis and tumor progression [27]. Dysregulated mitophagy also promotes stemness, as parkin-dependent mitophagy increases the expression of stem cell marker CD44 in cancer cells undergoing EMT. Levels of protein/mRNA expressions involved in mitochondrial dynamics, canonical and non-canonical mitophagy pathways in CRC patient samples were analysed. It has been found that Opa1, pDRP1, BNIP3 were upregulated and DRP1, CL, PINK1 were downregulated [28]. Diminution of UVRAG and decline of Bif-1 compromised autophagosome formation thereby dysfunctional autophagy, resulting in increased cancer cell proliferation in the colon, gastric, breast, and prostate cancer [3].

Autophagy in cancer is mainly mediated by Sestrin2 which protects the cells from cellular stress conditions such as oxidative stress, hypoxia, and glucose deprivation. Sesn2 mainly mediates autophagy through various signalling pathways such as Nrf2/Keap1, AMPK/mTORC1, etc. [29]. Disabled/dysregulated autophagy may promote tumor progression which constitutes a general hallmark of developing cancer. Dual roles of autophagy have been reported by p53, an on cosuppressor protein. Overexpression of p53 plays an important role in the regulation of autophagy. It stimulates autophagy through the transcriptional mechanism and inhibits autophagy when in the cytoplasm by inhibiting AMPK and activating mTOR. Depletion, deletion, or inhibition of p53 triggers autophagy in normal as well as in cancer cells [30]. The role of autophagy in cancer cells confers stress tolerance and serves to maintain tumor cell survival. Increased levels of autophagy were reported in human pancreatic cancer cell lines [31]. *In vitro* analysis of the combination of rapamycin and S-allyl-mercaptocysteine (SAMC) on HCT116 CRC cell lines showed increased levels of the expression of autophagy-related protein, LC3-II, thereby regulating autophagy. Western blot analysis and q-PCR results suggested that co-treatment of rapamycin and SAMC on HCT116 showed elevated levels of Nrf2 expression, thus activating an antioxidant system by regulating p62 [32]. Another study showed that treatment of tributyltin (IV) ferulate (TBT-F) on HCT116 cell lines, induced ER stress by ROS production, thereby evoking Nrf2 mediated antioxidant response. TBT-F treatment also increased the levels of LC3-II and p62 autophagic markers [33]. Glutaminase 1 (GLS1) is a key enzyme in glutamine metabolism that is known to show elevated levels in tumor and rapidly proliferating cells. Evidence shows that overexpression of GLS1 was observed in human malignancies and may embark tumor progression through activation of PI3K/AKT, MEK1/ERK1/2, or RhoGTPase signalling pathways. GLS1 is upregulated in CRC cell lines (HT29, HCT116, SW480, DLD-1, and NCM460). Thus blocking of GLS1 leads to inhibition of CRC cell proliferation and migration through a monitoring redox/Nrf2/autophagy pathway [34]. Benzyl isothiocyanate (BITC) is known to inhibit cell proliferation in CRC cells. It has been reported that BITC induces autophagy in human cancer cells. This was proved *in vitro* when BITC treatment enhanced LAMP1, LC3B-II, and p62 (autophagic proteins) in a dose- and time-dependent manner, thereby showing upregulation of Nrf2 and Keap1 downregulation, thus inducing autophagy in CRC (HCT116) cell lines [35]. *In vivo* analysis of effects of melatonin on mouse models of colitis-associated colon carcinogenesis (CACC) showed increased expressions of Nrf2 and associated antioxidant enzymes. Thus, it decreased the CACC progression by downregulating autophagy, which was correlated with expression levels of autophagic markers Beclin-1, LC3B-II, and p62 [36]. Epigallocatechin-3-gallate (EGCG) treatment on CRC cell lines (HCT116) showed increased levels of expressions of Nrf2, inducing Nrf2 translocation to nucleus and autophagy. EGCG treatment combined with radiation on HCT116 cells expressed increased sensitivity to radiation, thereby inhibiting cell proliferation, and

a significant increase of mRNA expression levels of LC3 and caspase-9 was observed [37]. Another study reported that metformin treatment on HT29 CRC cells inhibited cell proliferation by increasing apoptosis/autophagy. The inhibitory effects of metformin were due to inhibition of transcriptional activation of Nrf2 by time- and dose-dependent manner [38]. Treatment of stress-induced SW620 and Caco-2 CRC cell lines with docosahexaenoic acid (DHA) inhibited cell proliferation in both cell lines. DHA also induced the nuclear translocation of Nrf2, thereby triggering an anti-oxidative response. This is due to changes in levels of expressions of genes involved in oxidative stress, protein folding, and autophagy [39]. *In vivo* studies on mice treated with azoxymethane/dextran sodium sulfate and tamoxifen showed loss of autophagic activity, exerting anti-colon cancer effects. Expressions of p53, unfolded protein response (UPR) and ER-stress related proteins upregulation were also reported [40]. Topotecan treatment on HCT116 and LS174T cell lines induced autophagy, mediated by p53 through the activation of Sestrin2 was reported [41].

Conclusion and Future Course of Work

This review provides insights into the potential roles of Nrf2/Sestrin2 in mediating autophagy in cancer, which was observed and proved in various *in vivo* and *in vitro* analyses. Though, this mechanism is predominantly seen in most types of cancer, more studies are required to prove this type of autophagic mechanism in the case of metastatic colon cancer.

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Conflict of Interest

The authors declare no conflict of interest.

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