Digestive and Liver Disease xxx (xxxx) xxx

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Oncology

Possible role of nuclear factor erythroid 2–related factor 2 in the progression of human colon precancerous lesions

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ARTICLE INFO

Article history:
Received 23 August 2021
Accepted 20 January 2022
Available online xxx

Keywords:
Colorectal cancer
Inflammation
Polyp adenoma
Reactive oxygen species

ABSTRACT

Background: Increased levels of oxidative stress/cell inflammation contribute to colorectal cancer (CRC) onset. Nuclear factor-erythroid 2-related factor 2 (Nrf2) and its controlled growth factor erv1-like (Gfer) gene regulate redox-sensitive and anti-inflammatory mechanisms, respectively, which can contribute to promoting cancer development.
Aim: We evaluated Nrf2 and Gfer RNA expression and Nrf2 protein expression in colon mucosa in order to establish their possible involvement in the early stage of CRC.
Methods: Forty subjects were enrolled after a histological evaluation of their colon biopsies. They included 20 subjects with a sporadic colorectal adenoma (SpCA group) and 20 without precancerous lesions (controls). Biopsy samples were processed for gene expression analysis and protein expression, using Real-time PCR and immunofluorescence confocal microscopy, respectively.
Results: Nrf2 and Gfer mRNA expression were significantly reduced ($p=0.007$ and $p<0.003$, respectively) in SpCA tissues compared to normal mucosa from controls. Furthermore, immunofluorescence analysis confirmed a relevant reduction of Nrf2 in SpCA tissue compared to normal tissue from controls.
Conclusions: Our data confirm the hypothesis that Nrf2 and Gfer expression may be involved in the initial hits contributing to the multistep process of colon carcinogenesis. Further larger studies are needed to confirm if Nrf2 and Gfer are potential risk/prognostic factors for cancer development.

1. Introduction

The close relationship between precancerous lesions of the intestine and colorectal cancers (CRCs) has been well established by long-term clinical observations [1,2]. Precancerous lesions refer to pathological changes closely related to CRCs, including colorectal adenoma and inflammatory bowel disease (IBD)-associated dysplasia [3]. The morphological classification into polypoid, flat and depressed lesions is now a rather sensitive indication for malignancy [4,5].

As occurs for other neoplastic processes, colon carcinogenesis is determined by various factors: hereditary genetic predisposition, microsatellite and/or genomic instability, epigenetic influences, and environmental factors such as dysbiosis, inflammation, apoptosis impairment, etc. [1].

In the past years, extensive research has suggested that reactive oxygen species (ROS) also play an important role in cancer development [6,7]. Under physiological conditions, ROS levels are maintained by endognous redox sensitive transcription factors regulating the expression of antioxidant genes, including nuclear factor-erythroid 2-related factor 2 (Nrf2) [8]. Nrf2 is a basic leucine zipper redox-sensitive transcriptional factor that plays a central

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https://doi.org/10.1016/j.dld.2022.01.131
1590-8658/

role in the regulation of antioxidant and/or detoxifying genes in various tissues [9]. Oxidative stress and the related increase of ROS levels are greater in neoplastic than in normal cells [10]. ROS can damage cellular proteins, lipids, and DNA, inducing metabolic pathways that lead to inflammation [11]. All together, these conditions give rise to fatal cellular lesions that, in turn, contribute to many human diseases, including cancer [11–19].

Based on these premises, in this study we evaluated the expression of Nrf2, at RNA and protein levels, and Gfer, at mRNA level, in the intestinal mucosa from patients with colorectal adenomas (SpCA group) or without proliferative lesions (controls), to establish whether there is an association between the expression of these two genes and the development of colorectal adenomas.

2. Materials and methods

2.1. Patients selection and study design

Consecutive subjects undergoing colonoscopy at the Gastroenterology Unit, Policlinic University Hospital, Bari, Italy, participated in this study.

Our study was carried out in compliance with the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval by the institution's human research committee (protocol# CE/4748). Moreover, this study was registered on ClinicalTrials.gov (registration# NCT03417258). All patients gave their informed consent.

At the time of endoscopy, histological evaluations were performed on colonic polyps in subjects showig sporadic colorectal adenomas (SpCA group) and intestinal normal mucosa in subjects without proliferative lesions (controls). After the endoscopic and histological evaluation, 40 subjects were enrolled, 20 with SpCA and 20 controls. All tissues underwent molecular biology studies by real time PCR (RT-PCR) and immunodetection evaluations by confocal microscopy analysis. The inclusion criteria were subjects of both sexes aged between 50 and 75 years, undergoing colonoscopy. This age range was adopted for several reasons: screening colonoscopies are prescribed specially in subjects over 50 years of age, when the probability to find neoproliferative lesions increases; patients with a family history of CRC frequently develop polyps at a younger age. The exclusion criteria were age $<$ 50 and $>$ 75 years, previous diagnosis of colon cancer or inflammatory bowel diseases (IBD), family history of hereditary intestinal tumors, ongoing infections, intake of pre- or pro-biotics, symbiotic, and antibiotics during the 12 weeks that preceded colonoscopy, creatinine clearance below 60 mL/min, decompensated liver disease.

2.2. Total RNA extraction, RNA preparation and first-strand cDNA synthesis

Tissue samples were taken and kept in RNA Later (Qiagen GmbH, Germany) to preserve mRNA. Total RNA was extracted using the RNeasy Mini Kit (Qiagen GmbH, Germany) according to the manufacturer's instructions. Final mRNA concentrations were estimated by ultraviolet absorbance at 260 nm. Aliquots of total mRNA (1 µg) were reverse-transcribed using random hexamers and the TaqMan Reverse Transcription Reagents (Applied Biosystems, Monza, Italy) with 3.125 U/µl of MultiScribe Reverse Transcriptase in a final volume of 50 µl. Real-time PCR was performed in 96-well plates on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Specific custom-synthesized oligos, synthesis scale 0.05 µmol, were purchased by Aurogene S.r.l. (Rome, Italy). For gene expression specific primer/probe, provided by Aurogene S.r.l. (Rome, Italy) were used (Nrf2 gene, Fw: 5′CACGGTCCACAGCTCATCAT 3′; Rw: 5′CACGGTCCACAGCTCATCA 3′; Probe: 5′ caagaacaactccaa 3′. Erv1/Gfer gene, Fw: 5′CCTGCCCCTCTTAGGTTTGG 3′; Rw: 5′ GGCCTCCCAGTACTCCTACA 3′; Probe: 5′ gaagacagcgggcc 3′).

Data collection and analysis were performed using the machine software. As previously reported [20], briefly, a two-step reverse transcription-PCR was performed using first-strand cDNA with a final concentration of 1 x Taq-Man gene expression Assay and 1 x TaqMan Universal PCR Master Mix. The final reaction volume was 50 µl. Each sample was analyzed in triplicate and all experiments were repeated twice. A non-template control (RNAase-free water) was included on every plate. The thermal cycler conditions were 2 min hold at 50 °C, 10 min hold at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. In the first instance, a standard curve and a validation experiment were performed for each primer/probe set. A series of six serial dilutions (20 to 0.1 ng/µl) of samples cDNA were used as a template for each primer/probe set. Standard curves were generated by plotting the threshold cycle (TC) number values against the log of the amount of input cDNA. Threshold cycle is the PCR cycle at which an increase in reported fluorescence above the baseline level is first detected. The average and SD of amount of target gene expressed were normalized to an endogenous reference and is relative to a calibrator and the relative quantification for each sample was plotted in a bar chart using Microsoft Excel software. The endogenous reference used in all experiments reported here was GAPDH. A normal sample was used as calibrator in all experiments. The assays were supplied as a 20-x mix of PCR primers and TaqMan Minor Groove Binder 6-FAM dye labelled probes with a non-fluorescent quencher at the 3′- end of the probe.

2.3. Histology and Immunofluorescence

To assess the absence of histological abnormalities or the presence of low- and high-grade dysplasia, biopsies from "normal" mucosa or tissue specimens from intestinal polps were embedded in paraffin, cut at 4-µm thickness, and stained with hematoxylin-eosin for light microscopy observation. Architectural organization was examined to distinguish tubular or villous adenomas, while nuclear-to-cytoplasmic ratio, basal orientation of the nuclei, and presence of goblet cells were the parameter used to differenziate low- from high-grade dysplasia.

Nrf2 was investigated by monoclonal mouse antibody cell signaling (Santa Cruz, CA, United States). Tissue sections were rinsed in PBS buffer with TWEEN 0.025% (Merck, Milan, Italy) for 10 minutes and incubated in a microwave oven (citric buffer pH 6.0, 10 minutes, 750 W) for antigen unmasking. They were then treated (2 hours, room temperature) in 10% goat serum and 1% bovine serum albumin-blocking solution (Merck, Milan, Italy). They were subsequently incubated with anti-Nrf2 antibody (Invitrogen, Life Technologies, Monza, Italy), diluted 1:200 at 4 °C overnight. Alexa 488 fluorescent-conjugated goat anti-mouse (Invitrogen, Life Technologies, Monza, Italy) at a dilution of 1:200 represented the secondary antibody. Nuclear counterstain was obtained using TOPRO-3 (Invitrogen-Molecular Probes, Life Technologies, Monza, Italy) for 10 min at room temperature. All sections were observed with confocal microscopy magnification. Ten well-oriented crypt/villous associations were selected for the analysis. Cells were counted at 400 x magnification, by confocal microscopy (Leica TSC SP2 confocal laser scanning microscope) [20].

2.4. Statistical analyses

Sample distribution of continuous variables was performed by evaluating the symmetry with the Skewness and Kurtosis tests, and the variables were expressed as mean ± standard deviations (SD) and compared by *t*-test. Statistical significance was set at p

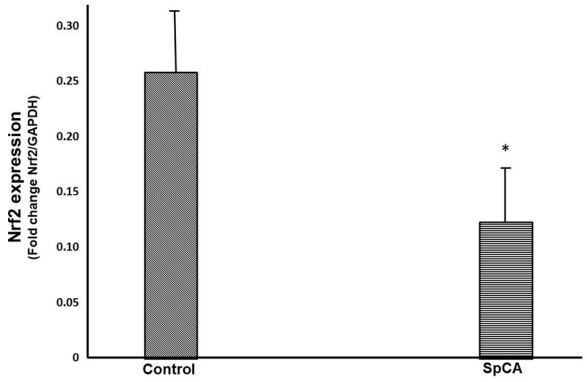

Fig. 1. Nrf2 mRNA expression in intestinal tissue from control and SpCA subjects. Nrf2: Nuclear factor-erythroid 2-related factor 2, SpCA: sporadic colorectal adenoma. Data espressed as mean ± SD represent the ratio between Nrf2 expression and the housekeeping gene expression (GAPDH). * p = 0.007.

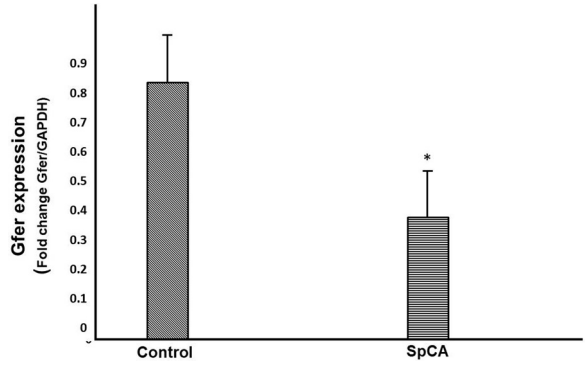

Fig. 2. Gfer mRNA expression in intestinal tissue from control and SpCA subjects. Gfer: growth factor erv1-like; SpCA: sporadic colorectal adenoma. Data espressed as mean ± SD represent the ratio between Gfer expression and the housekeeping gene expression (GAPDH). *p < 0.003.

<0.05. If not specifically reported, results were representative of at least three different experiments. The analyses were performed using Excel (Microsoft Corporation).

3. Results

Among the subject paticipating in this study, the gender ratio (M/F) in the 20 patients and 20 controls was 14/6 and 11/9, respectively (p=0.61), while their age was 63.4 ± 8 and 64.5 ± 8 years, respectively (p=056). Most polyps were tubular adenomas and only a few villous adenomas; in any case, only aspects of low-grade dysplasia were observed (data not shown). Fig. 1 describes Nrf2 mRNA expression in tissue samples from control and SpCA subjects. As shown in the figure, a statistically significant reduction of Nrf2 expression was observed in SpCA tissue compared to normal mucosa tissue obtained from controls (p = 0.007). Similarly, we found a statistically significant reduction of Gfer mRNA expression in tissue samples from SpCA subjects compared to control subjects (p < 0.003) (Fig. 2).

Fig. 3 describes the results obtained by confocal microscopy after the immunodetection of Nrf2 protein in controls (A-C) and SpCA tissues (D-F). Panel A highlights the nuclear counterstain of colonocytes by TOPRO-3; in panel B, the use of specific fluorescent-conjugated antibodies identifies the presence of Nrf2; panel C represents the merging of the two previously described panels, demonstrating the colocalization of Nrf2 in the nuclei (red arrows). A striking difference is observed in SpCA tissue (panels D, E and F) compared to controls (A-C panels). In particular, the image reported in panel D shows the presence of smaller and bundled nuclei, while in panel E there is a complete absence of Nrf2, which is obviously confirmed by the merging of the two images in panel F.

4. Discussion

Colorectal cancer is the third most commonly diagnosed cancer worldwide, representing 10% of all cancer diagnoses. In terms of mortality, CRCs is the second most deadly cancer worldwide, causing about 9.4% of deaths [21]. Most sporadic CRCs are not related to genetic predisposition or family history and are preceded by a benign neoproliferative lesion, either an adenomatous or a serrated polyp [5,22]. According to the "Big Bang" model proposed by Davis et al., of the many benign neoproliferative lesions, only those "born to be bad", i.e., endowed with peculiar genetic/functional properties and multiple genetic abnormalities, can evolve to CRCs [22]. Unfortunately, the molecular events or microenvironment situations that steer the polyps to this fate are still under evaluation [23–25].

In the present study we analyzed Nrf2 and Gfer expression in intestinal tissues from patients with precancerous lesions (SpCA group) and patients without proliferative lesions (controls). Our results clearly demonstrate a significant reduction of Nrf2 gene and protein expression, which was associated with a significantly reduced expression of Gfer in the SpCA group compared to controls. These results support the hypothesis that a reduced antioxidative and anti-inflammatory activity could play a pathogenetic role in the development of precancerous lesions, predisposing, as cumulative risk, to progression to CRCs. In fact, in most cases CRCs follow the adenoma-carcinoma sequence [24]. In these pathological situations, chronic inflammation and increased turnover of epithelial cells contribute to the progress of low- and high-grade dysplasia which may give rise to CRCs. Oxidative stress and the related ROS generated by an inflammatory infiltrate are thought to contribute to this process [25]. Our results are only apparently in contrast with literature data since it has been proved that NRF2 acts either as a tumor suppressor gene or as an oncogene [26]. Indeed, even if the protective role of the Nrf2 against carcinogenesis has been well documented in normal cells, oncogene (K-Ras, B-Raf, or Myc)-induced Nrf2 transcription would favor cancer growth by exerting a protective effect on neoplastic cells [27]. In addition, this pro-carcinogenetic behavior could be explained either by mutations of NRF2 or its upstream controller KEAP1, responsible of a hyperactivity [26], or by epigenetic modification of Nrf2 [28,29].

Under physiological conditions, oxidative stress triggers up-regulation of the endogenous antioxidant and cytoprotective proteins. Nrf2 is the master transcriptional regulator of genes encoding many antioxidant and detoxifying enzymes through its binding to EpRE/ARE [30–35].

An important demonstration of the role of Nrf2 in the intestinal carcinogenesis comes from the $APC^{Min/+}$-$Nrf2^{-/-}$ knockout mice model [36]. Using this experimental model, it has been demonstrated that the reduction of Nrf2 expression leads to the loss of cytoprotection due to diminished antioxidant capacity, lowered β-oxidation of fatty acids and, conversely, heightened sensitivity to ROS-based signal of apoptosis [37],[38]. Moreover, Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium (DSS)-induced inflammation in the colorectum leading to colitis and carcinogenesis [37], compared to wild-type mice. These data reinforce the evidence on the role of inflammation, lipid peroxidation, and metabolic dysfunction in CRC onset and development [39–41].

Interestingly, Nrf2 is able to induce Gfer gene expression , which encodes for the augmenter of liver regeneration (ALR) gene, as the latter is able to reduce DNA/protein damage and enhance cell survival [42–46]. Liver-specific ALR knock-out in mice (L-ALR-KO mice) have a reduced anti-oxidative cell capacities, resulting in

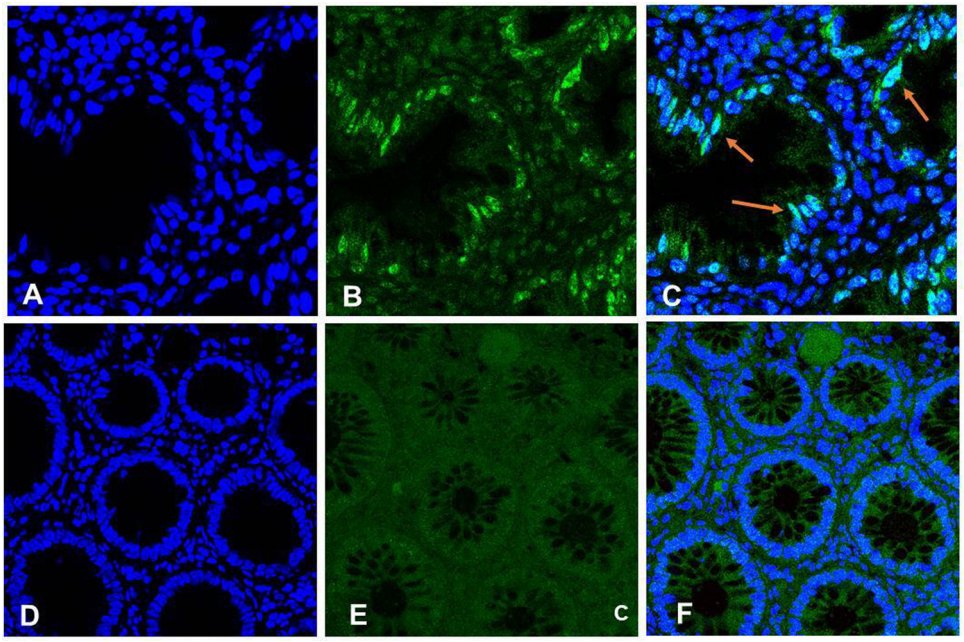

Fig. 3. Immuno-identification of Nrf2 in intestinal tissue samples from Control (3A-3C) and SpCA (3D-3F) subjects.
Nrf2: Nuclear factor-erythroid 2-related factor 2, SpCA: sporadic colorectal adenoma. A high immune signal is present in control tissues (3B) compared to SpCA tissues (3E), also revealing a nuclear presence of the regulatory factor (arrows in 3C).

an enhancement of ROS production and mitochondrial dysfunction, contributing to the develop of liver tumours [47]. Moreover, there are several reports that demonstrate the presence of ALR in colonic cells [48–51], further reinforcing our hypothesis on the role of anti oxidant and anti inflammatory factors in CRC development.

Our study has some limitations: the preliminary character of the study with the limited number of subjects did not allow us the possible relationship between the histological classification of adenoma and the degree of Nrf2 expression; however, the intent of our study was satisfied since we demonstrated a significant decrease of Nrf2 and its controlled Gfer gene in the precancerous lesions, suggesting their possible role as an additional prognostic factor to include in the future histological evaluations.

5. Conclusion

Considering the roles played by oxidative stress and cellular inflammation in the initiation and promotion of colon carcinogenesis, the present preliminary data support the hypothesis that the reduction of anti-oxidative and anti-inflammatory defenses controlled by Nrf2 and Gfer could contribute to the carcinogenetic process. In this contest the evaluation of Nrf2 and Gfer expression could represent new possible biological markers to include in the evaluation of colonic adenomatous lesions in order to implement preventive strategies. Future studies including colonic adenomatous lesions at different evolutionary stages are necessary to confirm the potential use of Nfr2 as marker/risk factor for CRC development.

Declaration of Competing Interest

None declared.

Funding

This research received no external funding.

Acknowledgements

None.

References

[1] Vodicka P, Urbanova M, Makovicky P, et al. Oxidative damage in sporadic colorectal cancer: molecular mapping of base excision repair glycosylases in colorectal cancer patients. Int J Mol Sci 2020;21:2473–93.
[2] Yang Y, Han Z, Li X, et al. Epidemiology and risk factors of colorectal cancer in China. Chin J Cancer Res 2020;32:729–41.
[3] Conteduca V, Sansonno D, Russi S, et al. Precancerous colorectal lesions (Review). Int J Oncol 2013;43:973–84.
[4] Sandouk F, Al Jerf F, Bassel Al-Halabi MHD. Precancerous lesions in colorectal cancer. Gastroenterol Res Pract 2013;2013:457901.
[5] Facciorusso A, Antonino M, Di Maso M, et al. Non-polypoid colorectal neoplasms: Classification, therapy and follow-up. World J Gastroenterol 2015;21:5149–57.
[6] Basak D, Nasir Uddin M, Hancock J. The role of oxidative stress and its counteractive utility in colorectal cancer (CRC). Cancers (Basel) 2020;12:3336–69.
[7] Gupta SC, Hevia D, Patchva S, et al. Upsides and downsides of reactive oxygen species for cancer: the roles of reactive oxygen species in tumorigenesis, prevention, and therapy. Antioxid Redox Signal 2012 Jun 1;16(11):1295–322.
[8] Prasad Kedar N. Simultaneous activation of Nrf2 and elevation of dietary and endogenous antioxidant chemicals for cancer prevention in humans. J Am Coll Nutr 2016;35:175–84.
[9] Nascimento EFR, Ribeiro ML, Magro DO, et al. Tissue expression of the genes MUTYH and OGG1 in patients with sporadic colorectal cancer. Arq Bras Cir Dig 2017;30:98–102.
[10] Meierjohann S. Oxidative stress in melanocyte senescence and melanoma transformation. Eur J Cell Biol 2014;93:36–41.
[11] Santacroce L, Bufo P, Gagliardi S, et al. Argyrophilic nucleolar organizer regions (AgNORs) as malignancy biomarkers in colorectal neoplasms. Clin Ter 2001;152:91–3.
[12] Prasad S, Gupta SC, Tyagi AK. Reactive oxygen species (ROS) and cancer: Role of antioxidative nutraceuticals. Cancer Lett 2017;387:95–105.
[13] Alexander A, Cai SL, Kim J, et al. ATM signals to TSC2 in the cytoplasm to regulate mTORC1 in response to ROS. Proc Natl Acad Sci USA 2010;107:4153–8.
[14] Benhar M, Engelberg D, Levitzki A. ROS, stress-activated kinases and stress signaling in cancer. EMBO Rep 2002;3:420–5.
[15] D'Autreaux B, Toledano MB. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol 2007;8:813–24.
[16] Fruehauf JP, Meyskens FL Jr. Reactive oxygen species: a breath of life or death? Clin Cancer Res 2007;13:789–94.
[17] Veal EA, Day AM, Morgan BA. Hydrogen peroxide sensing and signaling. Mol Cell 2007;26:1–14.
[18] Tudek B, Speina E. Oxidatively damaged DNA and its repair in colon carcinogenesis. Mutat Res 2012;736:82–92.

[19] Sheth S, Farquhar DR, Schrank TP, et al. Correlation of alterations in the KEAP1/CUL3/NFE2L2 pathway with radiation failure in larynx squamous cell carcinoma. Laryngoscope Investig Otolaryngol 2021;6:699–707.
[20] Principi M, Di Leo A, Pricci M, Scavo MP, et al. Phytoestrogens/insoluble fibers and colonic estrogen receptor β: Randomized, double-blind, placebo–controlled study. World J Gastroenterol 2013;19:4325–33.
[21] Xu P, Xi Y, et al. Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol 2021 Oct:101174.
[22] Barone M, Scavo MP, Papagni S, Piscitelli D, et al. ERβ expression in normal, adenomatous and carcinomatous tissues of patients with familial adenomatous polyposis. Scand J Gastroenterol 2010;45:1320–8.
[23] Davis A, Gao R, Navin N. Tumor evolution: Linear, branching, neutral or punctuated? Biochim Biophys Acta 2017;1867:151–61.
[24] Sievers CK, Grady W, Halberg R, et al. New insights into the earliest stages of colorectal tumorigenesis. Exp Rev Gastroenterol Hepatol 2017;11:723–9.
[25] Al-Sohaily S, Biankin A, Leong R, et al. Molecular pathways in colorectal cancer. J Gastroenterol Hepatol 2012;27:1423–31.
[26] DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, et al. Oncogene induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature 2011;475:106–9.
[27] Kang KA, Piao MJ, Kim KC, Kang HK, Chang WY, Park IC, et al. Epigenetic modification of Nrf2 in 5-fluorouracil-resistant colon cancer cells: involvement of TET-dependent DNA demethylation. Cell Death Dis 2014;5:e1183.
[28] Zhao XQ, Zhang YF, Xia YF, Zhou ZM, Cao YQ. Promoter demethylation of nuclear factor-erythroid 2-related factor 2 gene in drug-resistant colon cancer cells. Oncol Lett 2015;10:1287–92.
[29] Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet 2014;383:1490 -02.
[30] Menegon S, Columbano A, Giordano S. The dual roles of NRF2 in cancer. Trends Mol Med 2016;22:578–93.
[31] Rogler G. Chronic ulcerative colitis and colorectal cancer. Cancer Lett 2014;345:235–41.
[32] Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol 2010;38:96–109.
[33] Li W, Kong AN. Molecular mechanisms of Nrf2-mediated antioxidant response. Mol Carcinog 2009;48:91–104.
[34] Yu S, Kong AN. Targeting carcinogen metabolism by dietary cancer preventive compounds. Curr Cancer Drug Targets 2007;7:416–24.
[35] Dinkova-Kostova AT, Talalay P. Direct and indirect antioxidant properties of inducers of cytoprotective proteins. Mol Nutr Food Res 2008;52:S128–38.
[36] Li W, Khor TO, Xu C, et al. Activation of Nrf2-antioxidant signaling attenuates NFkappaB-inflammatory response and elicits apoptosis. Biochem Pharmacol 2008;76:1485–9.
[37] Wakabayashi N, Slocum SL, Skoko JJ, et al. When NRF2 talks, who's listening? Antioxid Redox Signal 2010;13:1649–63.
[38] Cheung KL, Lee JH, Khor TO, et al. Nrf2 knockout enhances intestinal tumorigenesis in Apc(min/+) mice due to attenuation of anti-oxidative stress pathway while potentiates inflammation. Mol Carcinog 2014;53:77–84.
[39] Khor TO, Huang MT, Kwon KH, et al. Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis. Cancer Res 2006;66:11580–4.
[40] Khor TO, Huang MT, Prawan A, et al. Increased susceptibility of Nrf2 knockout mice to colitis-associated colorectal cancer. Cancer Prevent Res (Philadelphia, Pa.) 2008;1:187–91.
[41] Polimeno L, Pesetti B, De Santis F, et al. Decreased expression of the augmenter of liver regeneration results in increased apoptosis and oxidative damage in human-derived glioma cells. Cell Death Dis 2012;3:e289.
[42] Dayoub R, Vogel A, Schuett J, et al. Nrf2 activates augmenter of liver regeneration (ALR) via antioxidant response element and links oxidative stress to liver regeneration. Mol Med 2013;19:237–44.
[43] Polimeno L, Rossi R, Mastrodonato M, et al. Augmenter of liver regeneration, a protective factor against ROS-induced oxidative damage in muscle tissue of mitochondrial myopathy affected patients. Int J Biochem Cell Biol 2013;45:2410–19.
[44] Lin S, Li Y, Zamyatnin AA Jr, et al. Reactive oxygen species and colorectal cancer. J Cell Physiol 2018;233:5119–32.
[45] Polimeno L, Pesetti B, Lisowsky T, et al. Protective effect of augmenter of liver regeneration on hydrogen peroxide-induced apoptosis in SH-SY5Y human neuroblastoma cells. Free Radic Res 2009;43:865–75.
[46] Cao Y, Fu YL, Yu M, et al. Human augmenter of liver regeneration is important for hepatoma cell viability and resistance to radiation-induced oxidative stress. Free Radic Biol Med 2009;47:1057–66.
[47] Vodovotz Y, Prelich J, Lagoa C, et al. Augmenter of liver regeneration (ALR) is a novel biomarker of hepatocellular stress/inflammation: in vitro, in vivo and in silico studies. Mol Med 2013;18:1421–9.
[48] Yan R, Zhang L, Xia N, et al. Knockdown of augmenter of liver regeneration in HK-2 cells inhibits inflammation response via the mitogen-activated protein kinase signaling pathway. Inflamm Res 2015;64:453–62.
[49] Polimeno L, Francavilla A, Piscitelli D, et al. The role of PIAS3, p-STAT3 and ALR in colorectal cancer: new translational molecular features for an old disease. Eur Rev Med Pharmacol Sci 2020;24:10496–511.
[50] Nguyen KH, Nguyen AH, Dabir DV. Clinical implications of augmenter of liver regeneration in cancer: a systematic review. Anticancer Res 2017;37:3379–83.
[51] Gatzidou E, Mantzourani M, Giaginis C, et al. Augmenter of liver regeneration gene expression in human colon cancer cell lines and clinical tissue samples. J BUON 2015;20:84–91.