Mini-Review

Fiber, cancer stem cells and the Wnt signaling continuum

Possibilities for colorectal cancer prevention and therapeutics

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Abbreviations: CRC, colorectal cancer; HDACi, histone deacetylase inhibitors; BCT, β-catenin Tcf; APC, adenomatous polyposis coli; SCS, stationary cancer stem cell; MCS, migratory cancer stem cell; EMT, epithelial to mesenchymal transition; MET, mesenchymal to epithelial transition

Key words: cancer stem cells, fiber, colorectal cancer, Wnt, butyrate, apoptosis, β-catenin, histone deacetylase inhibitors

Wnt signaling is implicated in the initiation of human colorectal cancer (CRC). While the relationship between Wnt activity and proliferation is well known, studies have also shown an association between high levels of Wnt activity and apoptosis. Through analyses of ten human CRC cell lines, we have found that inhibitors of histone deacetylases (HDACis), such as butyrate, promote apoptosis of CRC cells through hyper-induction of Wnt signaling; thus, hyper-activation of Wnt activity by HDACis may represent a novel preventive and therapeutic strategy against CRC. Our findings may explain why studies on the protective role of dietary fiber and its product butyrate against CRC have yielded inconsistent findings. We believe that the variability in the levels of induced Wnt activity and apoptosis in CRC cells observed in vitro reflects the in vivo existence of CRC subtypes with variable degrees of responsiveness to butyrate. Given that Wnt activity has been linked to intestinal stem cell renewal and the migratory potential of CRC stem cells, this essay will discuss the potential of modulation of Wnt signaling by HDACis as a therapeutic approach against CRC stem cells. Finally, we consider possible gene therapy approaches that target Wnt-positive CRC stem cells.

Introduction

Colorectal cancer (CRC) is potentially amenable to a high degree of prevention through dietary modulation. Consistent with such an approach, two large clinical studies have reported a protective role of dietary fiber against colon cancer; thus, a 40% decrease in colon cancer risk occurred at a mean dietary fiber intake of 35 g/day. In contrast to these reports, several studies have reported no relationship between fiber intake and colon cancer incidence. These different findings have been attributed to a lack of assessment of potential confounders (e.g., dietary factors other than butyrate). However, even when major confounders are taken into account, there still has not been a consensus about a relationship between dietary fiber and colon cancer risk.

The effects of dietary fiber on the incidence of colon cancer have been attributed to several factors, including the fermentation of fiber to butyrate by colonic microflora. Butyrate, an inhibitor of histone deacetylases (HDACi), produces cell cycle arrest, differentiation and/or apoptosis of CRC cells in vitro. Based upon studies conducted in our laboratory on the effects of butyrate on CRC cells in vitro, we have proposed the existence of different colon cancer subtypes as major factors as contributors to the variable findings on colon cancer risk and fiber intake. Furthermore, we have speculated that the efficacy of butyrate as a stimulator of the apoptosis of CRC cells derives from the ability of this agent to modulate Wnt signaling along a continuum of Wnt activity, with different steps along this continuum corresponding to varying effects on the CRC cell phenotype. Finally, we believe that CRC stem cells represent a critical target of potential HDACi therapy. In this essay, we discuss how modulation of Wnt signaling, by dietary or pharmacological, or gene therapy approaches, represents potential methodologies for CRC prevention and/or therapy. We also outline how the relationship between fiber, butyrate and Wnt signaling can explain the different findings in studies on the association between dietary fiber intake and CRC risk.

Wnt Signaling Continuum, Dietary Fiber and CRC Risk: The Influence of CRC Subtypes

A common characteristic of the majority of CRCs is the constitutive aberrant activation of the canonical Wnt signaling pathway due to mutations in the APC, β-catenin and axin genes; this aberrant activation is believed to be the initiating event in colonic tumorigenesis. Butyrate, likely a mediator of the protective role of fiber against colon cancer, hyper-induces canonical Wnt activity in
CRC cells such as SW620; subsequent analyses of ten human CRC cell lines treated with butyrate have established a linear causative relationship between the fold induction of Wnt transcriptional activity and the degree of apoptosis in the cell population.\(^9,21\) CRC cells that responded to butyrate treatment with a high level of induction of Wnt signaling exhibited a strong apoptotic response,\(^{21}\) and repression of the butyrate-induced increase in Wnt activity, through the use of dominant negative Tcf4,\(^{17}\) suppressed the high levels of apoptosis induced by butyrate,\(^{21}\) demonstrating a causative relationship between Wnt activity and apoptosis.

How can these findings be reconciled to the known effects of Wnt signaling in promoting cell proliferation? We propose that Wnt signaling through β-catenin/Tcf exists as a cellular gradient, within which suppression of Wnt activity leads to terminal differentiation/apoptosis; whereas, low to moderate levels of Wnt signaling promote cell proliferation and relatively high levels induce apoptosis (Fig. 1).\(^{12}\)

Our in vitro studies on ten human CRC cell lines with different mutations that deregulate Wnt signaling have identified two classes of CRC cell lines: those which respond to butyrate treatment with a high fold induction of canonical Wnt activity and apoptosis and those which exhibit a relatively lower fold induction of canonical Wnt activity and apoptosis.\(^{21}\) Studies on the suppression of clonal growth of CRC cells exposed to butyrate have revealed a continuum in their response to butyrate; within this continuum, relatively high fold induction of Wnt activity correlates with a relatively great suppression of clonal growth, and a lesser degree of induction of Wnt activity results in a lesser suppression of clonal growth.\(^{21}\) It is reasonable to assume that CRC cells in vivo similarly exhibit differential responses to butyrate. Therefore, the lack of a consistent association between fiber intake and colon cancer risk in epidemiological studies\(^1\)\(^{-5}\) are likely due to the fact that only a subset of colonic neoplasms respond to butyrate by hyper-induction of Wnt signaling and apoptosis.

The different findings observed both in vivo and epidemiological studies on the effects of butyrate on intestinal tumorigenesis can also be explained in part by variations in the level of intestinal butyrate derived from differences in experimental protocols or to variations in the butyrate-producing microflora between individuals and/or population groups.\(^{11,22,23}\) Prolonged exposure of CRC cells to relatively low levels of butyrate in vitro selects for cellular populations resistant to the effects of butyrate on Wnt activity and apoptosis.\(^{10}\) Therefore, in early stage adenomas, relatively low levels of intestinal butyrate may select for pre-malignant cells unresponsive to the apoptotic effects of butyrate, such that at a later stage, advanced adenomas and malignant tumors would primarily consist of butyrate resistant cells. Our in vitro findings suggest that only continuous high levels of dietary fiber and its product butyrate, begun at or before the earliest stages of colonic tumorigenesis, induce substantial induction of Wnt activity and subsequent apoptosis of abnormal cells. However, the possible existence of CRC stem cells in primary tumors with different sensitivities to butyrate and other HDACis suggests that agents of this kind can be effective even in the later-stages of malignancy, if the stem cells of the tumor remain HDACi sensitive.

**Wnt Signaling Modulation and CRC Stem Cells**

Cancer stem cells represent a subpopulation of neoplastic cells that exhibit the defining characteristics of stem cells; i.e., the ability to self-proliferate and to differentiate into varied cell types. Cancer stem cells presumably can give rise to tumors and are responsible for metastases and relapse, the latter occurring when treatment eliminates the bulk of relatively differentiated tumor cells, while leaving the cancer stem cell population relatively unaffected.

The “migrating cancer stem cell” (MCS) concept of Brabletz et al.\(^{25}\) may influence the hypothesis of a relationship between Wnt signaling and CRC progression. These authors make a distinction between stationary cancer stem (SCS) cells, which are anchored in epithelial tissue and do not migrate, and MCS cells, which possess the same proliferative and differentiating potential as the SCS cells, but also exhibit a migratory phenotype. MCS cells are derived from their stationary counterparts by acquiring a transient epithelial to mesenchymal transition (EMT), which allows them to disseminate. This dissemination can either be short-range, resulting in expansion of the primary tumor, or long-range through blood and/or lymphatic vessels, resulting in metastases. Once established in a new location, these cells may undergo a reversal of the EMT, or a mesenchymal to epithelial transition (MET), which reverses the growth arrest (and resistance to apoptosis) that characterizes the EMT phenotype. This MET ultimately results in enhanced proliferation, differentiation and growth of the metastases.\(^{25}\)

Wnt activity is intimately involved in these processes, as this signaling pathway has been linked to both stem cell generation and maintenance,\(^{26,27}\) as well as to the establishment of the EMT (reviewed in ref. 25). Several Wnt target genes promote the stem cell phenotype, among them survivin, which induces proliferation while inhibiting apoptosis, while another set of Wnt target genes, such as SLUG, L1CAM and LAMC2, are associated with EMT.\(^{25}\) The first set of “stemness” genes are expressed early in colonic tumorigenesis and likely require low levels of constitutive Wnt activity; the expression of these genes is maintained throughout the process of tumorigenesis. However, EMT-related genes are transiently upregulated in the invasive-front cells; these are likely MCS cells that express high levels of nuclear β-catenin. EMT-related gene expression is subsequently downregulated during MET, leading...
Targeting CRC Stem Cells

We believe that, HDACis, including butyrate derived from dietary fiber, can prevent CRC during the earliest stages of CRC initiation. However, it is possible that CRC stem cells, particularly MSC cells with higher levels of Wnt activity, can be effectively targeted by HDACi-induced hyper-activation of Wnt signaling even during the latter stages of tumorigenesis. Targeting MSC cells would be of great value as these cells cause metastases and therefore pose the greatest risk to the patient. In addition, any methodology that also targets SCS cells would be of significant benefit. Elimination of both the SCS and MSC CRC cells is clearly necessary for any therapeutic intervention against CRC to be truly curative (Fig. 2).

We have developed a Cre-Lox Wnt targeted gene therapy model system, which efficiently and specifically targets the expression of cell death inducing effector genes in Wnt positive CRC cells in vitro. Levels of expression of these genes can be finely regulated by the choice of promoter and by the use of pharmacological and genetic factors. Thus, it may be possible to utilize a promoter that has a specific threshold of activation, so that the effector gene will only be expressed in the presence of the relatively high levels of Wnt activity found in MSC cells, leaving normal colonic stem cells unaffected.

In some cases, hyper-activation of Wnt activity may not be the most efficient treatment of choice; for example, in some patients, early stage CRCs may be resistant to the effects of butyrate derived from dietary fiber. Utilizing dominant negative Tcf4 as an effector in a Cre-Lox therapy system, we have demonstrated that it is possible to downregulate Wnt signaling in a manner dependent on initial high levels of Wnt activity, suggesting a method for suppressing the heightened levels of Wnt signaling that produce EMT and result in the development of MSC cells (Fig. 2).

Conclusion

Determination of the in vivo relevance of the Wnt signaling continuum to intestinal tumor phenotypes will require the use of rodent models of CRC. In addition, it is important to determine if human CRC subtypes, analogous to the in vitro CRC cell lines that differ in their response to HDACis, exist in vivo. CRC cell lines derived from tumors of patients with a history of high dietary fiber intake would likely be less sensitive to the effects of butyrate (or other HDACis) than cells derived from colonic tumors of patients with a dietary history of low fiber intake; presumably, this is due to the differentiation of cells which exhibit lower levels of nuclear β-catenin.

Thus, consistent with our hypothesis that a continuum of Wnt signaling activates different sets of genes involved in decisions of colonic cell proliferation, metastasis and/or migration, it appears that “stemness”-promoting Wnt-target genes exhibit a low threshold of activation by Wnt signaling, while expression of the EMT-promoting Wnt target genes requires a higher level of Wnt activity. Therefore, along the Wnt activity gradient, alterations in CRC stem cell physiology directly result from changes in Wnt signaling, that are above or below certain threshold levels of activity required for EMT or MET.

The following scenario, as outlined by Brabletz et al., and based upon our own findings (reviewed in refs. 11 and 12), is plausible. Mutations in the Wnt signaling pathway result in low levels of nuclear β-catenin, inappropriately activating genes responsible for maintenance of the colonic stem cell compartment, and giving rise to SCS cells that self-renew independent of the normal stem cell microenvironment. SCS cells then proliferate and differentiate, leading to the development of adenomas, in which most of the cells are more differentiated and in which SCS cells constitute a small minority. With progression to late adenomas and carcinomas, levels of nuclear β-catenin in SCS cells at the tumor-host boundary are increased, activating the EMT gene expression profile and giving rise to MSC cells, which then migrate (reviewed in ref. 25). After migration, MET occurs and the process of tumor growth and further EMT, metastases and MET repeats itself for the duration of the disease process. Variation in the levels of Wnt activity are fundamental to changes in CRC stem cell physiology; therefore, modulation of Wnt activity, through pharmacological or genetic means, represents a potentially effective option for targeting CRC stem cells.
selection for butyrate resistance. CRC cell lines can be established from tumors of patients with relatively high and low dietary fiber intake; cells from these tumors can be tested for the degree of induction of Wnt activity and apoptosis after HDACi treatment. Furthermore, the role of hyper-activation of Wnt activity with respect to CRC stem cells will depend upon advances in the characterization of these cells, as well as confirmation of the validity of the migratory CRC stem cell concept and dissection of the gene expression profiles in CRC stem cell subtypes. Finally, experiments utilizing appropriate rodent models are required to establish the in vivo efficacy of our Cre-Lox Wnt-targeted gene therapy model system.

Acknowledgements

The work from our group described herein was supported in part by grants from the American Institute for Cancer Research and the National Foundation for Cancer Research.

References