

Reviewer Comment 1:

The current manuscript is look like a mini-review but not so-called to be an editorial. This is a hot topic to describe the CRISPR/Cas9 to explore the genes editing in inflammatory bowel disease. The current manuscript is poor preparation on the references citation. All of the references need to be cited from 1 to 42 on the text. At the same time, missing references citation such as 2, 3, 5, 10, 16, 17, 18, 19, 20, 21, 22, 31, 43, 44 in the body of manuscript and lacking of references 43, 44, 45 and 46 in the section of reference. It should not be accepted with the current status.

Response:

We are very grateful for your meticulous review of our manuscript and your invaluable feedback. Given the nature of the manuscript, we agree that its content may be a mini-review. In regards to the references cited, given the multiplicity of resources with no intention at all, we overlooked the congruency of our references for which we have corrected and painstakingly reviewed our references to reflect your input.

Addition in manuscript:

All references where cited and missing references were added.

Reviewer Comment 2:

When reading about inflammatory bowel diseases, it is important to know that Crohn's disease is not the same thing as ulcerative colitis, another type of IBD. The symptoms of these two illnesses are quite similar, but the areas affected in the gastrointestinal tract (GI tract) are different. Crohn's most commonly affects the end of the small bowel (the ileum) and the beginning of the colon, but it may affect any part of the gastrointestinal (GI) tract, from the mouth to the anus. Ulcerative colitis is limited to the colon, also called the large intestine. Crohn's disease can also affect the entire thickness of the bowel wall, while ulcerative colitis only involves the innermost lining of the colon. Finally, in Crohn's disease, the inflammation of the intestine can "skip"-- leaving normal areas in between patches of diseased intestine. In ulcerative colitis this does not occur. I ask question to author. 1. Please tell me the etiology which CRISPR/Cas9p cause good effect for UC and CD. 2. What kind of stage of UC and CD will be suitable for CRISPR/Cas9 therapy?

Response:

This is an excellent point that you make and you note a very important distinction between Crohn's disease and Ulcerative Colitis.

1. Given the shared similarity in symptomatology of these two illnesses, the disease etiology and the GI location it affects is different. However, In Crohn's disease, commonly a defective processing of intracellular bacteria, autophagy or innate immunity is the inciting factor. In Ulcerative Colitis, genetic evidence demonstrates genes that are responsible for proper barrier function are important in preventing UC. Furthermore, when analyzing genetic data in more detail, Crohn's disease and UC have shared disease

susceptibility and shared genetic gene profile. Specifically, for patients who have Crohn's disease, CRISPR/Cas9p may target genes involved in autophagy, processing of intracellular bacteria or innate immunity. And for patients who have UC, genes involved in barrier function may be targeted for good effect. Also, the genes we are suggesting in table 1 will shed light on disease susceptibility and give us insight to further our understanding of etiology, which CRISPR can cause optimal effect.

2. This is a great question; in fact we are grateful for the input so we can include the therapeutic timing in our manuscript. It is very important to consider the stage of UC and CD that will be suitable for CRISPR therapeutic intervention. However, given that CRISPR just recently became widely used, there are very few or no studies that show precise intervention timing using CRISPR, thus this manuscript will guide researchers what genes to target and seek the best timing for therapy.

Addition in manuscript:

Potential Therapeutic Timing

Crohn's disease and Ulcerative Colitis in general have three stages of disease progression, mild-moderate; moderate-severe; and severe. Currently there are no studies indicating potential therapeutic timing when to target affected genes using CRISPR in IBD, however several multicenter trials conducted administering human recombinant IL-10 during active mild/moderate stage of CD or during refractory CD as well as patients undergoing curative ileal or ileocolonic resection.^[43,44] However, results did not show significant clinical improvement or higher remission rates secondary to too low IL-10 dose and adverse effects of medication. In addition, IL-10 alone failed to effectively suppress variety of dysregulated proinflammatory cytokines.^[43,44] In later stages of disease process, significant dysregulation of proinflammatory cytokines and redundant pathways occur, thus single target impact is futile. Given that CRISPR can simultaneously multiplex several genes, it will aid researchers to devise appropriate intervention timing.^[46] We also suggest early intervention is optimal to prevent progression of disease and reduce complications. It is imperative to conduct studies to best identify role of CRISPR in various stages of disease.

Reviewer Comment 3:

This manuscript provides an overview on a current topic, including a discussion of limitations. Please check the correct spelling of medical terms; e.g., "Streptococcus pyogenies" -> Streptococcus pyogenes (Introduction, paragraph 4), "Interleuking 12" -> Interleukin 12 (Legend for Table 1). Language polishing is recommended; e.g., "Quickly evolving advancement of DNA engineering such as CRISPR technology and its exponential use in diseases such as sickle cell or HIV resistance in human embryos may suggest that its optimal time to further our understanding of IBD pathogenesis and treatment (Introduction, paragraph 5)"; "The deaminase region can be altered so that it mutates specific DNA bases, ie: C to T, or introduce a 'stop' codon in a specific place" ("Current Status"); "This allowed researchers to wipe out gene's function and determine

what gene is implicated in disease, but it's been difficult to fix a 'point mutations', which are majority of disease-associated human genetic variants" ("Limitations"); etc.

Response:

Thank you for your careful review, the spelling errors were inadvertently overlooked and the errors have been corrected. We also implemented your suggestion to polish the language in noted sections and the changes are reflected in the manuscript.

Addition in manuscript:

"Streptococcus pyogenes" in introduction paragraph 4.

"Interleukin 12" in Legend for Table 1

"CRISPR DNA engineering has advanced quickly and is evolving rapidly. It has already been used in diseases such as sickle cell or HIV resistant human embryos. Given its exponential application in disease variants, it may suggest that it's an optimal time to further our understanding of IBD pathogenesis and treatment. "

"The Cas9 protein has a deaminase region that may be altered to increase highly specific alternation of genome sequence, which will allow for broader specific DNA bases manipulation. ^[9]"

"It allows for researches to perform gene knockout studies, which help them, determine what gene is implicated in disease. However, since majority of diseases are associated with "point mutations", it is difficult to target and repair a mutated gene."