Women’s Health and CRISPR: Novel Cervical Cancer, Endometrial Cancer, Condyloma, Endometriosis, Fibroids and PCOS Diagnostic and Treatment Tools with CRISPR Nucleic Acid Approaches

In Women’s Health, there are conditions in which alternative or novel approaches could make diagnosis more accurate and make treatments more patient specific. With the discovery of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and Cas9, there are new tools to target specific sequences of DNA and RNA to create novel ways to diagnose and/or treat disease [1-6]. The 2020 Nobel Prize in Chemistry was awarded to Drs. E. Charpentier and J. Doudna for their discovery of CRISPR [1-4]. CRISPR, as initially described, was an in vitro technology that allowed DNA sequences to be targeted and cut at sequence-specific DNA sites. This technique used a combination of an RNA molecule as a sequence specific guide and a nuclease, Cas9 that cuts DNA. Since the initial discovery, advances have led to the ability to use CRISPR to target both DNA and RNA for possible diagnostic and treatment purposes. For CRISPR treatments, there are two possible strategies: ex vivo treatment in which the primary target cells are obtained from the patient, then treated with CRISPR constructions using viral vectors, electroporation or liposomes, then the treated cells are allowed to grow, and then are reinfused into the patient. Alternatively, an in vivo approach could be used in which the CRISPR molecules are delivered directly to the patient. There are still ethical concerns and drawbacks associated with CRISPR. A major ethical concern is the use of CRISPR to introduce germline genetic modifications in human embryos and to implant the embryos [4], which will not be considered here. CRISPR limitations include (1) inefficient rates of uptake of the CRISPR/Cas9 components; (2) the inaccurate nature of the CRISPR targeting which results in off target gene editing; and (3) Cas9 is from a bacterial source, which results in possible inflammatory response by the recipient. Cas9 antibodies have been found to be present in some human serum [4].

GAPS THAT NEED TO BE ADDRESSED

CRISPR technology is currently used and/or could be used for the following disorders, cervical cancer, endometrial cancer, condylomas, endometriosis, fibroids and PCOS. Cervical and endometrial cancer may be where CRISPR technology is initially used [3, 5-7]. Cervical cancer is most commonly caused by prior infection with high-risk HPV, like HPV 16 and 18. Viral genes E6 and E7 target p53 and Rb pathways, respectively, to result in cell cycle progression and oncogenesis of the affected cells. The reactivation of TP53 and PRb by knocking out E6 and E7 using CRISPR technology results in apoptosis of the cancer cells. There are clinical trials where CRISPR is used to target the HPV 16/18 E6 and E7 genes. Questions to be asked include the following: How to best use CRISPR for cervical cancer? Can CRISPR be made to be more general for cervical cancer? Endometrial cancer affects over 50,000 women per year. Progesterone, acting through the progesterone receptor, induces differentiation, promotes apoptosis and inhibits infiltration. Kavlashvili et al. [7] demonstrated that overexpression of the progesterone receptor results in the downregulation of Myc and Myc-related genes. Myc is highly expressed in endometrial cancers and is one of the drivers of cancer growth. For endometrial cancer, how can CRISPR up express progesterone receptors or target Myc expression? Can endometrial hyperplasia be a CRISPR target for treatment?

Condyloma (venereal warts) from low-risk HPV infections, such as HPV 6 or 11, is another area in which CRISPR could be used [8, 9]. Current treatments for condyloma include trichloroacetic acid, cryotherapy, laser treatment or surgery. Liu et al. [8] used CRISPR in HPV transfected keratinocytes to inactivate the viral gene E7, which is associated with uncontrolled proliferation that leads to condyloma development. By silencing HPV 6/11 E7 with CRISPR/Cas9, there was inhibition of cell proliferation and induction of apoptosis. CRISPR/Cas could also be used as a rapid method for nucleic acid detection of HPV [9]. For CRISPR in condyloma, one can ask the following questions: What is the best delivery vehicle for CRISPR for condylomas? What is the optimal treatment size of the condyloma? Can the CRISPR application be a one-time use?

Endometriosis is another possible disorder to use CRISPR [10, 11]. Endometriosis patients suffer from infertility and from pelvic pain. Up to 25% of patients with infertility have endometriosis. A decrease of a microRNA, loss of mir-Let 7B, has been found in patients with endometriosis, with mir-Let 7B also found in endometriotic tissue. Sahin et al. [10] found that intraperitoneal treatment in a mouse model with mir-Let 7B decreased the degree of endometriosis. Tapmeier et al. [11] studied families with endometriosis and found that endometriosis diagnosis is associated with Neuropeptide S receptor 1 (NPSR1). There was an association of low frequency missense coding variants of NPSR1 with familial, predominantly ASRM stage III/IV endometriosis. A blocker of NPSR1, SHA68R, was found in their mouse model to be associated with reduced pain and inflammation. Questions using CRISPR technologies include the following: Could CRISPR be used to manipulate mir-Let 7B levels and thereby treat endometriosis? Could NPSR1 be repaired or the expression of NPSR1 be modified using CRISPR technology so that patients with endometriosis have decreased symptoms?

Uterine fibroids are another disease that could be addressed using CRISPR [12, 13]. Uterine fibroids are found in up to 70% of women and are associated with abnormal uterine bleeding, pelvic pain and infertility. Up to 70% of fibroids have mutations of the gene for a mediator of transcription 12 (MEDI12). This gene product is a key subunit of RNA polymerase II transcription function. It appears that MEDI12 mutation is associated with transcription enhancer defects that occur through AP-1 downregulation, which then results in extracellular matrix dysregulation, which is a hallmark characteristic of fibroids. The following are
CRISPR questions that could be addressed: Could CRISPR/Cas be used to diagnose patients with MED12? Could CRISPR/Cas be used to correct the AP-1 downregulation found in uterine fibroids? Could CRISPR be used to correct epigenetic changes found in fibroids, such as dysregulated microRNAs, long noncoding RNAs or histone?

Finally, CRISPR technologies could be applied to PCOS [14, 15]. Patients with PCOS have problems associated with infertility and abnormal menstrual cycles. In addition, one of the associated findings for PCOS patients is that 70% have insulin resistance. MicroRNA, such as mir-206, dysregulation is associated with PCOS findings in steroidogenesis and ovarian morphology. Mir-206 has been found to be downregulated in PCOS. Zhou et al. [14] showed that mir-206 decreases CCND2 and induces granulosa cell apoptosis by regulating caspase 3 activity. Chen et al. [15] found previously unreported genes that are associated with insulin resistance. One of their described genes that has roles in adipogenesis, lipid metabolism and insulin signaling is follistatin (FST). Follistatin has been reported to be associated with the diagnosis of PCOS. In addition, follistatin binds to activin and serves as an activin antagonist. CRISPR questions to consider for PCOS include: Could CRISPR used to help diagnose the specific PCOS or insulin resistance defects in these patients? Could CRISPR be used to functionally rescue the abnormalities (such as follistatin) as a new approach to correct the physiologic abnormalities associated with PCOS?

CONCLUSION

CRISPR technology is a new tool that allows both novel diagnostic and therapeutic approaches to human diseases. Though initially considered useful in addressing and correcting DNA disorders, the additional ability of CRISPR to address and to modify RNA will lead to new diagnostic and therapeutic approaches to common gynecologic problems. Cervical cancer, endometrial cancer, fibroids, PCOS and condyloma all are currently being studied as Women’s Health disorders in which CRISPR may offer a new avenue to help patients in a more specific way with their gynecologic problems.

REFERENCES


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