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A Potential Function in DNA Damage Responses and a Reliable Tool for Translational Cancer Research: Phosphorylated H2AX

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Abstract

The grouped consistently interspaced short palindromic rehashes CRISPR related protein 9 (CRISPR-Cas9) utilized for genome altering. The utilization of CRISPR-Cas9 in quality altering is confronted with specific limits including askew change, diminished homologous recombination (HR) fix, and safe framework reactions. It appears to be that assuming Cas9 communicated in an inducible way, off-target transformations might diminish. The P53 protein diminishes the action of the HR pathway in the cell cycle, thus, the decline in P53 articulation level might build the action of this pathway. In light of this subject, interestingly, we planned "px601-Turbo GFP-TRE-shRNA P53" as a CRISPR-based vector. The utilization of this vector can at the same time initiate articulation of Cas9 and closure briefly P53 articulation under an inducible advertiser and an inciting specialist. Along these lines, closure fleetingly P53 might be prompting diminished off-targets and expanded precision of genome altering. In the human gastric disease MKN45 cell line, the P53 quality communicates at a typical level. Besides, CD44 in this cell line has overexpression and is a gastric malignant growth undifferentiated organism marker. To assess this speculation, CD44 will be focused on for a particular succession change (altering) by the px601-Turbo GFP-TRE-shRNA P53 vector. As needs be, in the wake of cloning and infection arrangement, MKN45 cell lines will be transduced within the sight of the proper doxycycline (DOX) dose. Eventually, to assess the vector effectiveness, DNA extraction and entire genome sequencing (WGS) will be finished and contrasted and the transduced MKN45 cells without an inducible guide and DOX as control bunch. Moreover, the Sanger sequencing for the objective quality should be finished. This transitory inducible articulation of P53 might seem to build the proficiency of the CD44 quality altering and diminish off-targets.

Keywords: CRISPR–Cas9; P53; HR Repair Pathway;CD44; Off-targets

Introduction

Gastric Cancer (GC) is one of the most reasons for cancerrelated casualty. Because of the helpful opposition and intricacy, GC must be treated in various methodologies. Gene treatment through bunched, routinely interspaced, short palindromic rehashes (CRISPRs), and CRISPR-related protein (Cas9) framework is novel with a high possible remedial methodology in the GC treatment. In this framework, as an endonuclease, Cas9 can target DNA destinations through guide RNA (sgRNA) and prompt Double Stranded Break (DSB) in the ideal objective DNA. DSBs actuate two significant sorts of DNA fix frameworks: 1) Error-inclined Non-Homologous End Joining (NHEJ) causes little inclusions and cancellations (indels), 2) HomologyDirected Recombination (HDR) prompts hereditary substitution by including exogenous contributor DNA successions [1]. The NHEJ pathway is worked at the G1 period of the cell cycle. While at the S or G2 stages, the Homologous Recombination (HR) framework is initiated, and ensuing CDK (cyclin-subordinate kinases) action forestalls NHEJ. P53 association in the HDR cycle would be hindered and kill G1 capture and deactivate the HDR framework. It has been exhibited that Cas9-determined DSBs initiate P53 and in this manner development capture at the G1 stage, which at last builds NHEJ and lessens HDR. Thus, cell cycle capture at the G1 stage might repress effective HDR by P53 capability, and on second thought, unsatisfactory fix might happen through NHEJ.4 as opposed to some GC cell lines, wild-type P53 communicates in MKN45 cells. CD44 is likewise a Cancer Stem Cell (CSC) marker in the MKN45 cell line that is overexpressed in this cell line. CD44 plays a significant part in the restorative obstruction of GC and its related mechanisms. In perspective on the abovementioned, we recommend with our vector plan, in the MKN45 cell line, it could be feasible to build the productivity of CD44 quality altering by P53 briefly repressing and diminishing off-targets through inducible Cas9 articulation [2].

Presentation of the Hypothesis

To research the job of transient concealment of P53 protein, we guessed a particular assignment of AdenoAssociated Virus-CRISPR (AAV-CRISPR). This vector can be utilized to prompt the statement of Cas9, and CD44 sgRNA Cas9-freely, which eventually prompts a significant accomplishment. At present, one of the restrictions of CRISPR-Cas9 is a consistent Cas9 articulation which prompts more askew mutagenesis. In addition, Cas9 is an extraneous antigen setting off have safe reaction that outcomes in the cancellation of the cells from the altered pool, hence influencing the proficiency and accuracy of the CRISPR-Cas9 device for altering the genome. Moreover, P53 articulation contribution in the HDR cycle would be restrained, prompting killing G1 capture and actuating the HDR framework. Many investigations have exhibited Cas9-inferred DSBs initiate P53 and in this way development capture at the G1 stage, which at long last builds NHEJ and decreases HDR. This is difficult for genome altering applications [3]. HDR is less normal than NHEJ and emerges just during S and G2 stages, though NHEJ emerges all through the phone cycle. HDR happens not fundamentally yet rather at the same time with NHEJ, and is uplifted in NHEJ imperfect cells. A viable

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method for approving precisely designated changes into the genome of mammalian cells by stifling the NHEJ key catalyst DNA ligase IV that will prompt quality redresses. Moreover, the NHEJ pathway can be stifled by hindrance of P53. Even however the P53 articulation restraint makes the cell defenseless against chromosomal adjustments and other tumorigenic transformations, a few examinations have showed no destructive cell modification, for example, carcinogenesis if there should arise an occurrence of transient hindrance of P53. Furthermore, utilizing even inducible P53 hindrance works on the drawn out endurance of mice. The inducible advertiser (TRE) of Cas9 and shRNA P53 is equipped for enlistment with a particular inducer for TRE temporarily. It has been shown that the transgenic mice with inducible CRISPR-Cas9 version by DOX treatment were effectively cloned. Therefore, it appears to be that the organization of DOX at lower focuses, ordinarily utilized with inducible articulation frameworks, meaningfully affected cell development [4].

Testing the Hypothesis

CD44 RNA guide grouping will be planned, combined, and cloned into this vector. Then, the bundling will give molecule infections and be transduced into the MKN45 cell line. DOX at lower focuses (10 μg/ml) can be utilized to actuate articulation of P53 and Cas9 autonomously and synchronic. Then, FACS will be utilized to distinguish adjusting GFP, and the RNP-transfected MKN45 cells will be refined. Following four days, recombinant clones will be chosen by puromycin or any marker situated in the vector. MKN-45 cell line transduced without an inducible guide and DOX) will be a benchmark group. Eventually, for productively assessing of the px601-Turbo GFP-TRE-shRNA P53 vector, the cell DNA of the cell gatherings will be removed independently. Then, the Whole Genome Sequencing (WGS) will be finished for the cell gatherings. The information will be examined and contrasted with explore whether the vector can prompt exact and further developed genome altering without aftereffects (off-focuses) in the phones [5]. As well as, the Sanger sequencing for the objective quality should be finished. The pace of marker articulation rectification in the two gathering cells can measure up by Real-Time PCR. In vivo examinations in creature models can likewise be made. The means are like in vitro approach.

PI3K-PKB (Phosphoinositide-3-KinaseProtein Kinase B)/ AKT Pathway Function of H2AX

The estimation of DNA harm set off because of maturing, deserts in DNA fix pathways and mutagenesis, frames the premise of clinical examinations. These triggers are answerable for cell destinies prompting senescence, corruption or apoptosis. The constant openness of the cell cycle to endogenous variables (hydroxyl extremist, nitric oxide and superoxide anion) and exogenous mutagens (UVB, ionizing radiation and synthetic compounds) make minor and repairable harm DNA. Be that as it may, the gathering of these harms throughout some undefined time frame might compromise the ordinary working of the phones. At times, the synthetic as well as actual variables might act together, and further, increase the degree of DNA sores. A few DNA fix components like Base Excision Repair (BER), Nucleotide Excision Repair (NER) and Mismatch Repair (MMR) are enacted in these occasions to forestall the legacy of harmed hereditary material by the descendants [6]. A few proteins including cyclins and cyclindependent kinases (cdk) are engaged with the execution of these systems. Hence, they go about as administrative proteins for legitimate progressing of the cell through resulting periods of the cell cycle. Thusly, the Double-Strand Breaks (DSBs) are one of the malicious DNA injuries which might happen because of a few physical, synthetic or natural elements. Openness to ionizing radiation is one of the normal elements prompting DSBs. Be that as it may, they may likewise happen, seldom, following a blunder during the replication or record cycles of the phone cycle. The DSBs prompts the phosphorylation of a histone variation H2AX, delivering γH2AX [7]. This, thusly, goes about as a prevalent component in enacting the DDR pathway as a guard system of an eukaryotic cell to recognize and fix the harm. The maintenance stage starts with the gathering of DNA fix proteins at the site of chromatin harm and cell cycle capture at a few designated spots. The practical proteins of the DDR pathway i.e., MRE11/NBS1/RAD50, MDC1, 53BP1, and BRCA1 cooperate with γH2AX to frame atomic foci. These DDR pathways, in this manner intercede the genotoxicity and replication stress by capturing cell movement and multiplication and starting DNA fix systems. The ATM, ATR and DNA subordinate protein kinases (DNA-PKs) are huge serine or threonine kinases that have a place with the PK family. They are the most upstream kinases and answer DNA harm by phosphorylating at the serine/threonine glutamine themes. They likewise direct a few designated spots following DNA harm. The DNA-PKs assume a part in enacting the primer NHEJ pathway. Then again, ATM and ATR proteins further actuate the designated spot kinases i.e., ChK1, ChK2 and MK2 by starting their phosphorylation. In particular, the DSBs enact the ATM, while ATR answers a more extensive range of DNA harm. Subsequently, the γ-H2AX, which is clearly seen in practically a wide range of DNA harm, has been recognized as a delicate biomarker for DSBs. The ID of explicit biomarkers is the underpinning of early conclusion, better forecast and the board of various kinds of malignant growths. The ongoing survey plays zeroed in with respect to H2AX in different DSB fix pathways and evaluation of DNA harm. An outline of the utilization of H2AX as biomarkers in the conclusion of different clinical problems is likewise introduced in this article [8].

Discussion

The PI3K-PKB/Akt is a profoundly moderated pathway that is controlled through multistep interlinked processes for its enactment. The class 1A PI3Ks invigorate the actuated receptors on restricting with their administrative subunit or connector atoms like Insulin Receptor Substrate (IRS) proteins. This interaction sets off the initiation of PI3K as well as the change of phosphatidylinositol (3,4)- bisphosphate (PIP2) lipids to phosphatidylinositol (3,4,5)- trisphosphate (PIP3). The PIP3 is then limited by PKB/Akt to start phosphorylation of T308 by PDK1 in the actuation circle. Thusly, the altered PKB/Akt actuates mTORC1 by straightforwardly phosphorylating and inactivating proline-rich Akt substrate of 40 kDa (PRAS40) and Tuberous Sclerosis protein 2 (TSC2). The actuated mTORC1 phosphorylates the ribosomal protein S6 (S6/RPS6) to advance protein combination and cell expansion. These instruments are reliant upon mTORC1 substrates like eukaryotic interpretation commencement factor 4E Binding Protein 1 (4EBP1), and ribosomal protein S6 kinase 1 (S6K1). The above occasions, in any case, lead to fractional enactment of the PI3K-PKB/Akt pathway. The total initiation happens on phosphorylation of Akt at S473 at the carboxy-terminal hydrophobic theme, either by mTOR or DNA-PK and intervenes a few cell capabilities like angiogenesis, digestion, development, multiplication, endurance, protein combination, record and apoptosis. It likewise represses the supportive of apoptotic FOXO proteins [9]. Here, each phone cycle is started by substrate-explicit phosphorylation occasions happening in the cytoplasm and core. The above occasions following DNA harm due to ionizing radiations or other mutagenic elements improve the DNA harm prompted record to help with cell endurance. The concealment of the Akt flagging pathway can happen because of dephosphorylating systems of different phosphatases acting at various locales. The practical phosphatases

like PHLPP1 and PHLPP2, dephosphorylate Atk at its hydrophobic theme site-serine 473 (S473). The dephosphorylation of Akt at its actuation circle (T308) and the hydrophobic theme is brought about by PP2A. The lipid phosphatase PTEN dephosphorylates PIP3 to change over it into PIP2 and subsequently eliminates the upstream sign for Akt initiation. The DNA-PK has been recognized as a putative S473 Akt kinase and ATM as its subset. They are gathered and confined alongside γ-H2AX and S1981 p-ATM at the site of chromatin harm. However the acceptance of Ser p-473 Akt has been accounted for to be autonomous of both PI3K and DNAPK. All things being equal, it happens downstream of Meiotic Recombination 11 (MRE11)-subordinate ATM initiation and Ring Finger Protein 168 (RNF168)-subordinate histone ubiquitinylation. Autonomously, the MRE11 complex (comprising of MRE11, RAD50 and NBS1) and RNF168 (an E3 ubiquitin ligase) capability as DSB sensor and γ-H2AX non-proteolytic polyubiquitinylation of the DSB-flanking chromatin individually, to reestablish the hereditary construction. Hence, albeit the capability of H2AX isn't totally found in the PI3KPKB/Akt pathway, it plainly assumes a significant part [10].

Conclusion

The determination of γH2AX levels is a sensitive biomarker for DDR and DSBs. This information has been potentially transformed into clinical opportunities in cancer assessment and therapy. The interaction of γH2AX with several proteins has been determined and these studies have immensely contributed to translational cancer research. The sensitivity of γH2AX to DNA lesions enables the evaluation of the biological effects of various anticancer drugs and radiations in low doses. In addition, γH2AX assays allow continuous monitoring to evaluate immediate as well as long term effects of these agents. Moreover, the threatened DNA damage anticipated on exposure to environmental pollutants and surrounding conditions can also be assessed based on the level of γH2AX. Hence early detection of unknown mutagens and DNA lesions can be treated at an early stage. One obvious disadvantage of γH2AX based studies is that anticancer treatment (with agents that induce apoptosis and DNA fragmentation of cancer cells) may result in increased stress and production of reactive oxygen species. This, in turn, stimulates the phosphorylation of H2AX. Although, cell death and anticancer treatment-related to H2AX phosphorylation can be discriminated against based on H2AX signals, they require the use of appropriate diagnostic techniques(fluorescence microscopy, cytometry) with suitable modifications to adapt to the different characteristics of cancer disorders.

Conflict of Interest

The authors declare that they have no conflict of Interest.

References

1. Zhang X, Wang A, Liu X, Luo J (2019) Dendrites in lithium metal anodes: suppression, regulation, and elimination. Acc Chem Res 52: 3223-3232.
2. Yin X, Zhang L, Harigai M, Wang X, Ning S, et al. (2020) Hydrothermal-treatment desorption of cesium from clay minerals: the roles of organic acids and implications for soil decontamination. Water Res 177: 115804.
3. Janica I, Del Buffa S, Mikolajczak A, Eredia M, Pakulski D, et al. (2018) Thermal insulation with 2D materials: liquid phase exfoliated vermiculite functional nanosheets. Nanoscale 10: 23182–23190.
4. Shao JJ, Raidongia K, Koltonow AR, Huang J (2015) Self-assembled two-dimensional nanofluidic proton channels with high thermal stability. Nat Commun 6: 7602.
5. Pan XF, Gao HL, Lu Y, Wu CY, Wu YD, et al. (2018) Transforming ground mica into high-performance biomimetic polymeric mica film. Nat Commun 9: 2974.
6. Alekseeva T, Alekseev A, Xu RK, Zhao AZ, Kalinin P (2011) Effect of soil acidification induced by a tea plantation on chemical and mineralogical properties of Alfisols in eastern China. Environ Geochem Health 33: 137-48.
7. Ogino R, Chinuki Y, Yokooji T, Takizawa D, Matsuo H, et al. (2021) Identification of peroxidase-1 and beta-glucosidase as cross-reactive wheat allergens in grass pollen-related wheat allergy. Allergol Int 70: 215-222.
8. Dale MP, Ensley HE, Kern K, Sastry KAR, Byers LD (1985) Reversible inhibitors of Beta.-glucosidase. Biochemistry 24: 3530-3539.
9. Kempton JB, Withers SG (1992) Mechanism of Agrobacterium beta.-glucosidase: kinetic studies. Biochemistry 31: 9961-9969.
10. Mattiacci L, Dicke M, Posthumus MA (1995) Beta-Glucosidase: an elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. Proc Natl Acad Sci 92: 2036-2040.