CRIPS Digest

Phase-separating peptides for direct cytosolic delivery for macromolecular therapeutics

Biotechnologically derived products such as peptides, recombinant proteins and mRNAs possess high target specificity, efficacy and lower risk profile; however they have poor aqueous solubility, stability and cellular permeability. To counter these pitfalls, various formulation techniques have been exploited. One of these novel strategies employ smart peptides that respond to physiological stimuli like pH, redox reactions, temperature, etc. The researchers used short Histidine-rich break peptide (HBpep) containing self-immolative moieties conjugated by disulphide bonds (HBpep-SR) in order to study the cytosolic delivery of macromolecules. HBpep was synthesized by the conventional Merrifield solid-phase peptide synthesis method, purified using HPLC and isolated by lyophilisation. The peptides so formed were modified by the addition of self-immolating moieties such as HO-SS-R and NHS-SS-R. These moieties initiated a chemical reaction between the e-amine terminal of the single Lys residue of N-terminal protected peptide and the amine-reactive species NHS-SS-R to form HBpep-K and HBpep-SR. The desired concentration of the Enhanced Green Fluorescence Protein (EGFP) was dissolved in a buffer (pH 7.5). A stock solution of peptides (pH=7.5) was mixed with EGFP-containing solution in a 9:1 ratio to form coacervates. The entrapment efficiency was quantified using a fluorescence spectrophotometer taking the supernatant buffer before and after coacervation. The mean size range of EGFP-loaded HBpep-K and HBpep-SR peptide coacervates was reported in the range of 964-982 nm with narrow size distribution. The zeta potential of peptide-coacervates was reported to be -12.4 mV. No potential cytotoxicity was reported with pristine coacervates and EGFP-loaded coacervates in the HEK293 cells. The redox-responsive behavior was screened by analyzing the EGFP release from the coacervates in the presence of glutathione (GSH) in HepG2 cells. These coacervates formed vesicular structures inside the cells. As soon as the pH was shifted from 7.5 to 6.5, the whole EGFP content was released within 24 h which confirmed the pH-responsive behavior of peptide coacervates. In the presence of a cytosolic compartment rich in GSH, the peptide moieties were reduced by GSH and the redox-responsive release of EGFP was triggered within 4 hours. To study the cell internalization mechanism, EGFP-loaded coacervates were incubated with HepG2 cells, and LysoTracker staining was performed. Confocal microscopy confirmed that the prepared coacervates did not localize into endosomes. The treatment of cell lines with endocytosis inhibitors did not reduce the internalization of coacervates. Coacervates loaded with mRNA were incubated with RNase to check if the prepared coacervates possess mRNA protective activity. The researchers reported that coacervate-loaded mRNA cargoes had a delivery efficiency of ~98% compared to 81% of free mRNA. The entrapped mRNA was protected by peptide coacervate from RNase, thus more efficient delivery was reported. Following these encouraging results, the scientists aspire to establish in vivo efficacy of the presented drug-delivery system to enhance its translational value. (Nat. Chem. 2022, 14(3): 274-283)

Mesenchymal stem cell-derived exosomes protect against liver fibrosis by delivering miR-148a to target KLF6/STAT3 pathway in macrophages

Nowadays, the Mesenchymal Stem Cells (MSCs)-based techniques are considered one of the best due to their unique characteristics and immunomodulatory effects. The applicability of MSCs as a regenerative solution for various diseases including spinal cord injury, organ fibrosis, inflammatory bowel disease and graft-versus-host disease is continuously emerging. Despite intensive research, the underlying mechanisms involved in the therapeutic potential of MSCs for liver fibrosis are still unexplored. Currently, MSCs-derived exosomes (MSC-EXOs) are widely accepted as crucial messengers for intercellular communication. In this particular study, researchers attempted to explore the therapeutic effects and mechanisms of MSC-

EXOs in liver fibrosis. Liver fibrosis was induced by carbon tetrachloride followed by intravenous injection of MSCs or MSC-EXOs to assess the therapeutic potential. The resulting histopathology, extent of fibrosis, inflammation and macrophage polarization were analyzed. The regulatory effects of MSC-EXOs on macrophage polarization were assessed by using RAW264.7 and BMDM cell lines. Then, the critical miRNA involved in the therapeutic effects of MSC-EXOs was identified by RNA sequencing and validated experimentally. Further, the target mRNA and downstream signalling pathways were elucidated by luciferase reporter assay, bioinformatics analysis and western blot. MSCs alleviated liver fibrosis by secreting exosomes which were found to be circulating into the liver after transplantation. Additionally, MSC-EXOs modulated the macrophage phenotype which then regulated the inflammatory microenvironment and repaired the liver injury. Mechanically, RNA-sequencing illustrated that MSC-EXOs enriched miR-148a targeted the Kruppel-like factor 6 (KLF6) to suppress the pro-inflammatory macrophages and promote anti-inflammatory macrophages by inhibiting the STAT3 pathway. Liver fibrosis was significantly reduced when miR-148a agomir or MSC-EXOs enriched with miR-148a were administered. Conclusively, MSC-EXOs may act as a potential therapeutic target for liver fibrosis by delivering miR-148a which normalizes intra-hepatic macrophage functions through KLF6/STAT3 signaling. (Stem Cell Res. Ther. 2022, 13(1): 330)

DNA damage assessment for precision medicine: an emerging diagnostic tool

DNA damage analysis as a diagnostic tool is a contributing factor in the development of precision medicine. The use of potential biomarkers such as phosphorylated histone 2Ax (?H2AX) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) has increased the growth in the field of DNA damage analysis. Several other novel biomarkers have been incorporated into routine patient categorization into cohorts with specific disease predispositions which allows early diagnosis of a disease. Advanced diagnostic technologies have been incorporated to evaluate a multitude of new possible biomarkers. Various approaches, features, technological advancements and prospective clinical applications of biomarkers of DNA damage in the context of precision medicine have been explored. Tumor heterogeneity is considered as a major obstacle to precision medicine. Much progress has been achieved in treating cancer patients based on the individual's diagnostic profile. A paradigm shift seems to take place where the high heterogeneity among the tumors is addressed by a more comprehensive biomarker-based identification and prediction in patients. For example, the fluorescence microscopy-assisted counting of ?H2AX foci, a marker of DNA damage, in the case of DSB assessment is getting popularity over other ?H2AX-detecting methods due to its superior sensitivity. Biomarkers of diffuse large B-cell lymphoma, one of the most aggressive forms of non-Hodgkin's lymphoma, have been diagnosed in more than 50% of lymphoma patients over the age of 65. A wide range of biomarkers would be required to enable complete and early detection of oncological illnesses. The utilization of capillary peripheral blood mononuclear cells as a potential substrate for the automated detection of "H2AX foci" is one of the most suitable diagnostic methods. There are a number of open-source image processing software programs like Cell Profiler, Icy, ImageJ/Fiji, and Find Foci, graphical user interfaces that are currently popular for counting H2AX foci. (J. Lab. Precis. Med. 2019, 4: 1-4)

Modelling metabolic diseases and drug response using stem cells and organoids

Metabolic diseases including obesity, diabetes mellitus and cardiovascular disease are the major threats to health in the modern world. Obesity is linked to non-alcoholic fatty liver disease (NAFLD) which eventually leads to non-alcoholic steatohepatitis (NASH). There is no FDA-approved therapy for NASH, but human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSCs) based technologies can generate various disease-relevant cell types. Animal models of metabolic diseases are limited due to unique human biology. Lack of proper animal models is responsible for the failure of drugs in the clinical trials. These failures are due to species specificity of the genomes and epigenomes. The goal of the research communities nowadays is to develop new models which replicate human pathophysiology and exploring the rational treatments, therapies and approaches. Organoids are 3D organ culture technologies to model human physiology and disease in biomedical research. Human adipose and liver organoid models are generated from healthy/diseased tissues and pluripotent stem cells such as hPSCs, ASCs and fully differentiated primary cells. Understanding the various cell-cell interactions through pluripotent stem cells techniques may lead to new therapeutic targets. Adipose organoids and spheroids have greater utility than classic adipose cellular models due to their greater morphological and functional resemblance to adipose tissue. LGR5+ stem cells generate organoids from other tissue stem cells. In the mouse liver, LGR5+ stem cells were

activated by carbon tetrachloride and cultured with Wnt agonist R-spondin. Human adult liver organoids were derived from EpCAM+ biliary cells and maintained genomic stability after long-term expansion. Hepatocyte organoids have high proliferative potential in-vivo. Adipose organoids can be used to model obesity and T2DM, while liver organoids can be used to model liver diseases that exhibit impaired cell-cell interaction and tissue structural alteration. Human organoid technology generates patient-specific organoid models. It can be used for drug discovery and precision medicine to understand the regulation and function of circadian clocks in humans. The use of individual-derived stem cells provides a platform for personalized medicine. High-throughput screening, precision medicine and organoid models can identify compounds promoting metabolic diseases. Advanced gene editing tools can prevent or rescue phenotypes of genetic diseases by repairing or inactivating mutations in cellular and animal models. CRISPR-Cas9 and base editing are used to revert a disease-causing mutation to wild-type in patient-derived hepatic organoids. Combining stem cell-derived organoids with gene editing and functional genomics has revolutionized the approach of finding treatments for metabolic diseases; however, it has been accompanied by many challenges and limitations such as heterogeneity and reproducibility. Ongoing development of synthetic and versatile scaffolds will reduce cost, increase reproducibility and promote the translational efficacy of organoids for future use. (Nat. Rev. Endocrinol. 2022, 18(12): 744-759)

The cGAS–cGAMP–STING pathway connects DNA damage to inflammation, senescence and cancer

Cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS) is a DNA sensor which is activated due to presence of DNA in the cytoplasm instead of nucleus. It has been reported that cGAS acts as a danger-associated molecular pattern (DAMP) to induce type I interferons (IFNs) and other cytokines through production of a second messenger cyclic GMP-AMP (cGAMP). Further, cGAMP binds and activates the adaptor protein STING which activates TANK-binding kinase 1 (TBK1) and IFN regulatory factor 3 (IRF3) along with activation of NF-?B. However, cGAS can be activated by double-stranded DNA irrespective of the sequence. Cytoplasmic DNA is a consequence of nuclear DNA damage that is observed in the form of micronuclei. These are the by-products of chromosome damage, centromere hypomethylation and kinetochore dysfunction which occurs due to genotoxic stress. Cytoplasmic DNA may accumulate in Trex1-deficient cells to activate cGAS. MUS81 is an endonuclease which converts nuclear DNA into cytoplasmic forms. The cGAS–cGAMP–STING axis connects DNA damage to auto-inflammatory diseases. Ataxia-Telangiectasia (A-T) is a consequence of dysfunctional V(D)J, a genetic recombination phenomenon, observed in vertebrates. Dysfunctional V(D)Jcan be due to the mutation in A-T kinase enzyme. Another neuronal disease Aicardi-Goutières syndrome (AGS) caused by a recessive mutation in trex1, rnaseH2a, rnaseH2b and samhd1 is also associated withan elevation in type I IFNs. cGAS is also altered in DNA damage-induced cellular senescenceand irreversible cell cycle arrest which is induced by a variety of external and internal stress such as telomere shortening, oxidative damage and oncogenic sign through the senescence-associated secretory phenotype (SASP). SASP can mediate both positive and negative effects of senescence in cancer. Cytosolic DNA activates the cGAS–cGAMP–STING pathway in APC along with activation of Type 1 IFN mediated by nuclear MUS81 activity which is responsible for anti-tumour immunity. cGAS–STING pathway acts as an intrinsic barrier to tumorigenesis by linking DNA damage to several antitumor mechanisms like NLRP3 inflammasome activation, immune surveillance, cellular senescence and cell death. On the other hand, it also activates cGAS–cGAMP–STING pathway which further promotes inflammation-driven carcinogenesis and metastasis. cGas–cGAMP–STING pathway can be a promising target with regard to cancer therapies like chemotherapy, radiation therapy and immunotherapy. (J. Exp. Med. 2018, 21(9): 1287-1299)