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Construction of tumour disease models based on CRISPR/Cas and iPSC technology

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Abstract. Cancer, which is defined as malignant tumours and neoplasms, is one of the most concerned diseases in the world. According to the WHO website, almost 10 million of people lost their lives due to cancer in 2020. The complexity of tumorigenesis and cancer development, which have not been explored enough, lead to its high morbidity and mortality. Therefore, it is in an urgent need to establish stable and reliable tumour disease models to study the complicated pathogenesis, hence developing novel therapies and drugs. As the third generation of gene-editing technology, CRISPR/Cas system has the advantages of high suitability to all types of model forms owing to the simplicity and convenience of operation. It can efficiently construct tumour disease models for verifying and screening related targets. iPSC, as a stem cell technology, can provide a new cell source for CRISPR/Cas system-based tumour disease modelling. It can be the basic of establishing disease models in specific areas like brain tumours. This review introduced the advantages of CRISPR/Cas gene editing and iPSC technology in establishing disease models and the combined application strategies of the two technologies through the cases of tumour disease modelling in the past five years. It also explored the modelling strategies and model categories adopted in the popular cancer field.

Keywords: CRISPR/Cas System, iPSC, Tumour, Disease Model.

1. Introduction

The study of tumour mechanism and cancer therapy have always been the key and difficult points in the development of human medicine, which are inseparable from disease models. In recent decades, with the development and improvement of research techniques in the field of molecular and cellular biology, many new ideas for establishing tumour disease models and developing future target sites and therapies have been generated. Among these technologies, CRISPR gene editing technology and induced pluripotent stem cell (iPSC) technology are landmark discoveries in the field of gene editing and stem cell respectively. They provide new methods for gene editing and new cell sources for establishing disease models.

This review illustrated how quite a few cancer models were established using CRISPR/Cas system and iPSC, separately or jointly. Cases from 2018 to 2023 were chosen to investigate the diversity of model forms and modelling approaches.

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2. CRISPR/Cas system

2.1. Mechanism of CRISPR/Cas system

Clustered regularly interspaced short palindromic repeats (CRISPR), is a gene editing technology which has been widely used in the past two decades. The CRISPR/Cas system, first discovered in bacteria, is used to recognize and cleave DNA fragments. It functions by identifying and cutting genomic targets to form double-stranded breaks (DSBs) by generating nuclear localization Cas9 protein, forming single guide RNA (sgRNA) complementary to the target sequence, and identifying protospacer adjacent motif (PAM) sites with sequences of NGG. The repair of DSBs depends on nonhomologous end-joining (NHEJ) and homology-directed repair (HDR). The former could introduce random DNA indels while the latter functions by adding a homologous DNA template at the DSB site [1].

In addition to the DSB-dependent CRISPR-Cas toolbox, high-precision single base editing can also be performed through base editing (BE) and prime editing (PE) without DSB. Epigenetic modification like knocking down or up the expression level of the target gene can also be achieved by using CRISPRi/CRISPRa [2].

2.2. Application of CRISPR/Cas system in tumour disease modelling

2.2.1. Liver cancer. Primary liver cancer is a kind of malignant tumour that occurs in liver or bile ducts. According to the cancer statistics in America, it had the second highest mortality from 2017 to 2022 [3]. Liver cancer models are needed to discover and verify specific targets and signalling pathways related to tumour cell development. For example, genome-wide screening using CRISPR/Cas9 system in hepatocellular carcinoma (HCC) cells was performed to discover that aldolase A (ALDOA) is a growth promoting factor of HCC under hypoxic conditions [4]. In another case, mouse models constructed by knockout (KO) of three specific promoters were used to explore the signalling pathway mechanism of circRNA CircIpo11 promoting the proliferation of liver cancer stem cells [5]. In addition, CRISPR screening in liver cancer models can study targeted therapy resistance and explore new immune checkpoints to develop new targeted therapies. For example, DOCK1 expression suppresses the effect of metformin, which is a potential anti-tumour drug [6]. And the expression of phosphoseryl-tRNA kinase (PSTK) inhibits ferroptosis during HCC chemotherapy [7]. Combination therapy can be carried out by inhibiting the expression of such loci.

2.2.2. Breast cancer. Breast cancer cells occur in the milk ducts and/or the milk-producing lobules of the breast. They show great ability of invasion and can spread into other tissues or organs. Breast cancer models, mainly in the field of triple-negative breast cancer (TNBC), are also used to discover and identify tumour-related loci such as oncogenes, tumour suppressor genes and immune checkpoints. For example, high-throughput CRISPR screening in TNBC mouse models was performed to determine the ability of Lgals2 overexpression to enhance immunosuppression, which is a potential immunotherapeutic target [8]. Considering the strong invasion of breast cancer, especially TNBC, determining tumour metastasis-related factors is the key to the treatment of breast cancer. In vivo whole-genome loss-of-function CRISPR screening was designed in a mouse model derived from circulating tumour cells (CTCs) of human breast to identify metastasis-related genes during in vivo disease progression, such as targetable genes IL18R1, ITGA2, CSNK1A1L and CSNK2A2. Their knockout reduced spontaneous metastasis by reducing CTC clusters [9]. For specific directional metastasis, such as lung metastasis, Kyle Lewis et al. established a mouse tumour model by injecting two cell models in vivo and determined that the expression of p66ShcA redox protein was a necessary but insufficient condition for lung metastasis [10].

Currently, CRISPR/Cas system has been widely used in solid tumour research, namely, to discover and verify tumour-related genes and tumour metastasis-related mechanisms, thereby improving existing therapies, developing new targets or combination therapies. In addition to developing specific therapies in specific tumour areas, high-throughput CRISPR screening in cancer cell lines can establish a

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correlation map of cancer therapeutic targets and determine their priority [11]. A target map for pediatric cancer was established by this means [12].

2.3. Advantages of CRISPR-based tumour disease models.

First and foremost, considering the simplicity and convenience of CRISPR gene editing technology, it can be applied into all types of model forms. It can be seen from the above cases that CRISPR-based tumour disease models are generally divided into cell models and animal models. Mice are often selected as experimental animals for animal models. CRISPR-based mouse tumour models are divided into genetically engineered mouse model (GEMM) and implantation model. GEMM is established by editing mouse embryos to express Cas-related proteins, which are then edited by delivery of sgRNAs. For example, the mouse model of TNBC can be created by expressing the hybrid protein of base editor BE3 and cytidine base editor (CBE) to identify sgRNA for base editing [13]. Such tumour disease models can be generated in mice to study the heterogeneity of tumour development, but may also fail to precisely regulate the timing of tumour formation. Implantation models, including ectopic model and orthotopic model, can also be edited by CRISPR/Cas system. Ectopic models are usually established by subcutaneous injection of edited tumour cells. It is easy to observe the growth and development of tumours or study physical characteristics of CRISPR-edited tumours like radiosensitivity [14]. The orthotopic model requires establishment of the tumour model at the tumour origin site in mice, taking into account the internal environmental factors. Hematologic tumours typically rely on this modelling approach. Shan Lin et al. used an orthotopic xenograft model to identify targets with high transformation potential in acute myeloid leukemia (AML), including the grow-supporting inositol transporter SLC5A3 and the E3 ligase MARCH5 [15].

In recent years, the emergence of organoids has brought a new direction for tumour disease modelling, broadening the application range of CRISPR gene editing. The generation of organoids can be induced by patient tumour culture or iPSC differentiation. And it is more convenient to directly apply patient-derived organoids for easily obtained tumours [16]. As mentioned above, to study the role of DOCK1 and metformin in the HCC model, four CRISPR-edited HCC organoids were established to test the inhibition of DOCK1 sensitivity to metformin in vitro [6]. It can be verified in combination with in vivo experiments based on mouse models. In addition, gastroesophageal junction (GEJ) adenocarcinoma has always lacked a suitable disease model. Hua Zhao et al. generated GEJ organoids through human primary endoscopic GEJ, which enabled CRISPR double knockout of TP53 / CDKN2A to evaluate its tumorigenicity [17]. Tumour organoids can also cooperate with high-throughput CRISPR screening. For example, human small intestine organoids were established and ten genes related to TGF-β drug resistance were screened using whole-genome CRISPR [18]. Compared with the 2D tumour cell model, the 3D tumour organoid model can highly restore the physiological characteristics of the internal environment. And the CRISPR screening in organoids is more accurate and stable.

To conclude, CRISPR/Cas system shows obvious advantages of simplicity and convenience. It can be used in not only the traditional models like mice but also the new ones. Moreover, CRISPR/Cas system can be applied for high-throughput screening of potential targets, which is more accurate and stable.

3. iPSC

3.1. Mechanism of iPSC

Induced pluripotent stem cells (iPSC) is a technology to generate pluripotent stem cells by introducing specific transcription factors to induce the reprogramming of adult cells. As a source of stem cells, they will not raise ethical issues compared with embryonic stem cells. More importantly, the discovery of iPSC means that cells that are difficult to obtain, such as cardiomyocytes, neuronal cells, and pancreatic β cells, can be induced to establish patient-specific in vitro disease models [19].

Besides creating hard-to-obtain cell models, iPSC can also be induced to form organoids. This 3D in vitro model can effectively restore the systematicity and complexity of tumour development in vivo,

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such as liver organoids. Given the tumorigenicity of iPSC, the diversity of liver tumours can be effectively studied [20]. iPSC induces differentiation that expands the cell source, which can not only establish brain organoids and other models that are difficult to obtain directly, but also provide organoids derived from healthy cells to generate controls in drug testing [16].

3.2. Application of iPSC in tumour disease modelling

The iPSC-based tumour disease models focus on brain tumours, hematologic malignancies, melanoma, and retinoblastoma, etc. Based on the complexity and fragility of the brain, brain tumours require nerve cells for modelling, which is iPSC-dependent.

Taking the field of Medulloblastoma (MB) as an example, iPSC-derived nerve cells are the first choice for modelling. For instance, Evelyn Susanto et al. obtained neuroepithelial stem (NES) cells from iPSCs derived from patients with Gorlin syndrome and healthy adult cells to establish the control group of MB model. LGALS1 was identified as the target gene of sonic hedgehog (SHH) [21]. This model has great potential in the screening of small molecule drugs for Gorlin syndrome and MB [22]. Yingchao Xue et al. used synthetic mRNAs coding the Atoh1 TF to generate neuron precursors (NPs) in iPSCs. This team infected NPs with lentiviral vectors to generate MYC/DNp53 co-expression to test the inhibitory effect of sea cucumber-derived Frondoside A on MYC-driven MB [23]. In summary, the case of MB established iPSC-based cell models and orthotopic models through patient-derived and lentiviral infection methods respectively. Brain organoids were used to study the global characteristics of tumorigenesis in the brain, such as the tumorigenicity of caudal late interneuron progenitor (CLIP) cell amplification. In this study, Oliver L. Eichmüller et al. used iPSCs derived from tuberous sclerosis complex (TSC) to construct human brain organoids as tumour disease models [24].

Similar to brain tumour models, tumour disease modelling in other fields is also based on cell models, mouse models and organoids. Often, the verification of tumour-related loci requires the establishment of iPSC models derived from healthy cells to generate control groups or repeated verification of two or more models.

3.3. Advantages of iPSC-based tumour disease models

As mentioned above, iPSC-based tumour disease models are mainly for diseases whose related cells are difficult to obtain. The ways to generate disease models are divided into two types: the first is to induce patient-derived cells to be reprogrammed as iPSCs and then differentiated into target models; the second is to differentiate iPSCs derived from healthy cells into target cells, and then induce oncogene expression by means of lentiviral infection. For different research purposes, different ideas can be selected, such as the MB disease models of different sources mentioned above [21-23]. The types of tumour models are not too specific, and all of them are centred on cell models, mouse models and organoids. Considering that the diseases targeted by iPSC-dependent models are relatively complex, joint verification of multiple models is required. Some non-solid tumour organoids are also in development, such as bone marrow organoids for hematologic malignancies. This team successfully simulated the bone marrow structure that supports the growth of primary blood cancer cells by mixing matrix glue, type I and type IV collagen and adding growth factors [25]. The establishment of new tumour disease models provides a multidimensional verification perspective and a deeper exploration space.

4. The combined application of CRISPR and iPSC in tumour disease modelling

The iPSC technology helps to establish CRISPR-based tumour disease models mainly in providing cell sources, while the CRISPR technology helps to establish CRISPR-based tumour disease models mainly in the convenience and efficiency of gene editing. In non-iPSC-dependent organoids modelling, some genetic disease models can accelerate the generation of transgenic organoids by using iPSC, such as kidney organoids related to autosomal dominant polycystic kidney disease (ADPKD) [26]. The establishment of hereditary breast cancer disease models caused by BRCA1 mutations by using mesenchymal stem/stromal cells (MSCs) derived from patient-derived iPSCs is referred [27]. The genetic related loci of cancer can be jointly studied by iPSC modelling from patients' family members.

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The differentiation of iPSCs to generate target cells requires mutations induced by means of lentiviral infection, which can be achieved by CRISPR. For example, the atypical teratoid/rhabdoid tumour (ATRT) model can be induced by CRISPR/Cas9 in human induced pluripotent stem cells (hiPSC) by knocking out TP53 and SMARCB1 [28]. The introduction of four mutations into iPSCs through sequential CRISPR editing has also successfully constructed models of three stages of clonal hematopoiesis (CH), myelodysplastic syndrome (MDS) and AML, and determined early therapeutic targets [29]. In addition, in iPSC-dependent disease models, especially in the field of non-solid tumours such as AML, the use of CRISPR technology can be used for high-throughput screening of targets and drugs.

5. Conclusions

Both CRISPR-based and iPSC-based tumour disease models can be divided into in vitro cell models and organoids, and in vivo models represented by mouse models. Among them, CRISPR-based tumour disease models can accurately and efficiently verify and screen tumour-related loci, while iPSC-based tumour disease models provide difficult-to-obtain cell sources. The combined application of the two can produce complementary effects. Some tumour areas that are difficult to obtain experimental cells can be modelled by iPSC. The obtained models can be screened by high-throughput CRISPR, so as to develop appropriate loci and drugs. As for establishing tumour diseases models that are iPSC-independent for CRISPR screening, control groups can be set up by using healthy-derived iPSC.

This review systematically introduced the mechanism of CRISPR/Cas system and iPSC technology, and their applications on construction of tumour disease models. CRISPR/Cas9 system and CRISPR derivative technology have been used to knock out specific tumour-related genes in cell models and mouse models for verification, or screens potential targets through the whole genome. Meanwhile, patient iPSCs can be derived to establish models, or induces healthy-derived iPSCs to develop oncogene mutations to form tumours.

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