Use of Human Pluripotent Stem Cells to Define Initiating Molecular Mechanisms of Cataract for Anti-Cataract Drug Discovery

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Abstract: Cataract is a leading cause of blindness worldwide. Currently, restoration of vision in cataract patients requires surgical removal of the cataract. Due to the large and increasing number of cataract patients, the annual cost of surgical cataract treatment amounts to billions of dollars. Limited access to functional human lens tissue during the early stages of cataract formation has hampered efforts to develop effective anti-cataract drugs. The ability of human pluripotent stem (PS) cells to make large numbers of normal or diseased human cell types raises the possibility that human PS cells may provide a new avenue for defining the molecular mechanisms responsible for different types of human cataract. Towards this end, methods have been established to differentiate human PS cells into both lens cells and transparent, light-focusing human micro-lenses. Sensitive and quantitative assays to measure light transmittance and focusing ability of human PS cell-derived micro-lenses have also been developed. This review will, therefore, examine how human PS cell-derived lens cells and micro-lenses might provide a new avenue for development of much-needed drugs to treat human cataract.

Keywords: human pluripotent stem cell; lens; micro-lens; cataract; bioinformatics; risk factor; regeneration; ROR1 cells; anti-cataract drug

1. Introduction

Cataract is a condition in which light transmission through the ocular lens is decreased, resulting in reduced vision and blindness. The ability to define the initiating molecular mechanisms of human cataract formation—and, therefore, effective treatments to inhibit or delay cataract progression—has largely been hampered by the lack of access to functional human lens tissue at the initial stages of cataract formation. The ability of human pluripotent stem (PS) cells to (i) self-renew and (ii) differentiate into any cell type of the body, means human PS cells can provide a large-scale source of normal or diseased human cells for research [1–4]. Consequently, human PS cells are enabling new research approaches into human cell and tissue development, elucidation of molecular disease mechanisms, drug discovery and toxicity assessments, and investigation of candidate cell-based therapies. This review will explore how human PS cell technology is being applied to cataract research, with particular emphasis on cataract disease modelling, drug discovery and toxicity assessment.

2. Human PS Cell-Derived Organoids

The types of human PS cells most widely used for research are embryonic stem cells [5,6] and induced pluripotent stem cells [7–9]. Cell culture maintenance of human PS cells involves non-trivial tasks compared to culture of non-pluripotent cell lines. This is due to human PS cells
being highly sensitive to variations in basic culture parameters, including the size of cell aggregates, cell and/or cell-aggregate density, time in culture, growth factor and extracellular matrix composition and concentrations, etc.

Significant efforts were made worldwide to identify effective proliferation and maintenance conditions for human PS cells. A comparison of published culture media by the International Stem Cell Initiative identified three media conditions capable of sustained maintenance of multiple human PS cell lines across five independent laboratories [10]. Nowadays, commercially available human PS cell media provide defined, feeder-free culture conditions for robust and reproducible expansion of human PS cells.

As a consequence of having reliable human PS cell maintenance conditions, human PS cell differentiation strategies are now being improved to the extent where generating large numbers of purified, differentiated cells is possible for a variety of cell types. Moreover, human PS cell differentiation strategies have begun to evolve to the point where they can reproducibly generate large numbers of small, three-dimensional human tissues, termed ‘organoids’. These stem-cell-derived organoids mimic aspects of the cellular arrangement, and to varying extents, the overall function, of human tissues [11–13]. Human PS cell-derived organoids, therefore, have the potential to provide new and powerful tools for elucidating molecular mechanisms of disease progression that are specific to individual disease risk factors, as well as associated drug discovery studies [14–16].

3. Human PS Cell-Derived Lens Epithelial Cells and Micro-Lenses

As summarized by Murphy et al., a number of methods have been used to produce lens epithelial cells (LECs) at different levels of purity from human pluripotent stem cells [17]. The method that generates the most purified LEC population involves cell purification via an antibody that detects the ROR1 (receptor tyrosine kinase-like orphan receptor 1) cell surface antigen. Subsequent aggregation and culture of these purified LECs generates micro-lenses that share key properties of primary human lenses, including:

(i) The ability to transmit and focus light;
(ii) A cellular architecture consisting of LECs and a mass of lens fibre cells;
(iii) Expression and accumulation of lens-specific crystallin proteins;
(iv) Ultrastructural changes, including lens fibre cell denucleation and formation of complex membrane interdigitations.

Of the various methods used to produce lens cells from human PS cells, the ROR1-LEC/micro-lens system shares the largest number of functional lens properties with primary human lenses, in particular, the ability to focus light. Accordingly, this review will focus on how the ROR1-LEC/micro-lens system might be used to investigate cataract formation.

To test whether human PS cell-derived micro-lenses might be suitable for investigating cataract formation in vitro, ROR1-expressing LECs were exposed to a drug (Vx-770) suspected of causing non-congenital cataract in young cystic fibrosis patients [18,19]. Strikingly, micro-lenses treated with high Vx-770 concentrations lost their ability to transmit and focus light [17]. These findings suggest human PS cell-derived, ROR1-expressing LECs and micro-lenses could aid identification of the specific, initiating cataract molecular mechanisms that result from different cataract risk factors. The ability to precisely alter the environment in which stem-cell-derived, human lens cells and micro-lenses are cultured—for example, by changing the concentration of oxygen (hypoxia, normoxia, hyperoxia), nutrients, drugs, etc.—provides a new opportunity to define how individual or combinations of factors lead to cataract initiation and progression.

4. Cataract: Impairment of Lens Function

The term cataract describes an opacification of all or specific regions of the ocular lens. Cataracts can cause reduced vision and blindness by impairing the lens’ ability to focus light onto the retina.
Various anatomical and molecular changes have been associated with cataractous lenses including brunescence, formation of light scattering particles, and localized changes in the refractive index [20–22]. These effects can reduce the luminosity, contrast and/or clarity (‘acuity’) of images being received by the retina. The magnitude of these effects depends on the cataract morphology and the proportion of the pupillary area occupied by the cataract (see Figure 1).

Figure 1. Diagram of the lens and cataract types. (a) Location of the lens, lens epithelial cells (LECs) and lens fibre cells within the eye. Black dots indicate nuclei within epithelial cells and differentiating fibre cells. (b) Location of different types of cataract within the lens, including anterior subcapsular cataract (ASC), posterior subcapsular cataract (PSC), cortical cataract (CC) and nuclear cataract (NC). (c) Location of posterior capsule opacification (PCO) in the lens capsular bag after cataract surgery. Lens epithelial cells undergo an epithelial-to-mesenchymal transition (EMT) and cause capsular wrinkling.

Cataracts typically occur in adults, though they can also occur in children—for example, congenital and traumatic cataracts. In adults, cataracts most often present slowly and painlessly. Due to the subtle progression of cataract formation, many patients are often unaware of the initial changes in their vision [23]. The gradual nature of cataract formation, together with the inability to access lens tissue at the early stages of disease initiation, has made identification of risk-factor-specific mechanisms of cataract formation highly challenging.

5. Types of Cataract

Cataracts can be defined by different characteristics, for example, their location within the lens.

- **Nuclear cataract** is located in the center (or ‘nucleus’) of the lens. With ageing, the lens nucleus can darken, changing from clear to yellow and even brown; a process called brunescence [24,25].
- **Cortical cataract** forms within the peripheral layers of lens fibre cells, situated outside of the lens nucleus. Cortical cataract (e.g., diabetic cataract) often has a wedge- or spoke-like appearance pointing towards the centre of the lens, and is frequently associated with glare [25,26].
- **Anterior subcapsular cataract** arises within the anterior LEC monolayer; it results from abnormal growth and/or differentiation of lens epithelial cells, resulting in fibrotic plaques [25,27].
- **Posterior subcapsular cataract** forms under the posterior lens capsule due to abnormal growth and differentiation of LECs or immature lens fibre cells [28]; it can cause light sensitivity and glare [25,29].
- **Posterior capsule opacification** (PCO) is the most common complication of primary cataract surgery. PCO develops from residual LECs not removed during primary cataract surgery. These cells proliferate, migrate and undergo abnormal differentiation on the posterior capsule, causing capsular wrinkling [30].
6. Cataract Surgery

Currently, cataract development cannot be delayed. However, cataracts can be surgically treated via phacoemulsification to remove the lens cells and associated cataract—this typically happens after significant loss of vision has occurred. Lens cell removal is followed by implantation of a rigid plastic intraocular lens that restores a fixed focal point onto the retina (Figure 1). Cataract surgery generally restores vision immediately, and so has become common place in developed countries where the required equipment and expertise are readily available. Vision restoration in cataract patients restores more than just the lifestyle and functioning capacity of the patient. Due to the care that cataract patients require as a result of their vision loss, vision restoration via cataract surgery often has wider effects on family members and caregivers—for instance, freeing time for family members to return to the workforce [31,32].

Millions of cataract surgeries are performed worldwide each year, costing billions of dollars. In the USA, for 2004, the direct medical costs attributed to cataract were estimated at $6.8 billion [33]. Cataracts also contribute to an estimated $8 billion in annual productivity losses per year in the USA [33]. Additionally, estimates suggest between $65 and $157 million is spent annually treating PCO [34,35]. Despite this large investment in cataract health services, cataract continues to be a leading cause of blindness worldwide, with the number of patients with low vision or blindness due to cataract having increased from ~50 million in 1990 to ~65 million in 2015 [36]. Additional, less frequent but large-impact complications of cataract surgery include: refractive error, retinal detachment and visual impairment [37,38]. As a result of these side-effects, there is significant patient, clinical, industry and academic interest in identifying effective anti-cataract drugs to delay cataract formation. Notably, it has been estimated that delaying cataract formation by 10 years could almost halve the number of cataract surgeries required [39]. At present, however, no effective anti-cataract drug has been identified for humans. This is largely due to the inability to access human lens material during the early stages of cataract formation.

7. Cataract Risk Factors

Cataracts are often categorized based on the location in which they develop within a lens, though it is unlikely the molecular pathology of cataract formation is the same for all cataract subtypes. While some later aspects of cataract formation may be common to more than one type of cataract—for example, light-scattering particles such as protein aggregates [40] or multi-lamellar bodies [41]—it is likely that at least some of the initiating mechanisms of cataract formation are unique to each particular cataract risk factor responsible for cataract formation.

Various environmental cataract risk factors have been identified, including age [42], heat [43,44], UV light [45–47], smoking [48], diabetes [49], oxidation [24] and some drugs, such as glucocorticoids [28]. Over 300 genetic mutations have also been associated with congenital cataract or adult cataract [50], including crystallin [51,52] and connexin mutations [53]. Partial molecular mechanisms have been postulated for some forms of cataract, such as congenital cataracts caused by connexin mutations [54] and age-related cataract [55,56].

Much of our knowledge of lens biology has come from in vitro and in vivo studies of animal lens and cataract development, including drosophila, mice, rats, dogs, cows, salamanders, rabbits, and kangaroo [24,57–69]. These animal-based studies have been valuable in providing a framework for understanding human lens and cataract biology. Nevertheless, animal models are poorly predictive of human biology [70–72]. While various animal models have been used to test the ability of different molecules to delay cataract formation [73,74], to date, no effective anti-cataract drug has been identified for human patients.

From a practical perspective, in vitro models for anti-cataract drug discovery benefit from the ability to be performed at a small scale (for targeted studies of drug candidates) to large scale (for drug screening assays). These requirements mean that whole animal lenses are poorly suited to many anti-cataract drug discovery approaches. In addition to scalability issues and inherent species-specific
differences compared to humans, in vitro animal-based cataract models—such as explanted lens tissue—often lack the three-dimensional arrangement of normal lens tissue. As a result, cataract models that rely on explanted lens tissue [75,76] may not mimic some important lens parameters that occur in vivo, such as lens capsule-mediated access of drugs to lens cells.

To try and avoid the limitations inherent to animal-based investigations of cataract formation [56], primary human lens material has been used [77]. This includes the human lens capsular bag model, emulsified lens material obtained through cataract surgery, and small numbers of donated human lenses. These studies have defined important differences between late-stage adult cataract and aged, non-cataractous lenses. This includes a role for the lens epithelium in maintaining lens health through ion transport/homeostasis, and subsequent circulation of anti-oxidants through the lens [55] that appears to be affected in late-stage cataract. Nevertheless, key aspects of lens circulation still need to be defined, including how cataract progression is affected by functional heterogeneity within the lens epithelium—and more research is needed. However, the small amount of primary human material that can be accessed—together with the late stage of cataractogenesis obtained and the irregular supply of primary human material—makes it challenging to use these models for defining initiating mechanisms of primary cataract formation. Immortalized human cell lines have been employed as an alternative [78,79], but how closely these cells reflect normal lens biology is debatable. Furthermore, human lens cell lines have not been shown to develop into transparent, light-focusing three-dimensional lens tissue—a key feature of normal lens biology.

8. Defining Cataract Mechanisms with ROR1-Expressing Lens Cells and Micro-Lenses

As described in Section 3, human PS cells can be used to generate large numbers of ROR1-expressing LECs and micro-lenses. Exposing these micro-lenses to clinically relevant doses of a potential cataract risk factor (Vx-770) reduced micro-lens transparency and focusing [17]. The ability to collect cell culture samples at any time after treatment suggests ROR1-expressing LECs and micro-lenses could provide valuable new systems for defining initiating mechanisms of PCO and primary human cataract. Being able to control which cataract risk factor or combinations of risk factors the lens cells and micro-lenses are exposed to could provide new insights into the initial stages of cataract formation—insights that cannot be obtained using mouse lenses or primary human lens tissue. For example, an interesting approach could be to study human PS-cell-derived micro-lenses that possess crystallin mutations, with and without exposure to environmental cataract risk factors. Defining the molecular consequences of cataract risk factors on functional human micro-lenses could potentially also provide new information on lens-protection mechanisms. These new insights could then lead to new candidate anti-cataract drug targets and/or anti-cataract drugs.

The variety of cataract risk factors available for modelling via stem-cell-derived LECs and micro-lenses (noted above) can be prioritized based on the complexity required to replicate particular risk factors in vitro. Currently, used drugs that have primary cataract as a side-effect might be the simplest cataract risk factors to investigate, due to the ease in which they can be added to culture media over a range of clinically relevant concentrations. For example, dexamethasone is routinely prescribed to treat a variety of disorders, such as rheumatoid arthritis [80], ocular inflammation [81], and post-surgical inflammation [82,83]. Notably, long-term use of dexamethasone has been associated with posterior subcapsular cataract [84–86]. Preliminary data from our group has shown that micro-lenses exposed to dexamethasone have reduced light transmission and focusing ability (Figure 2). These data suggest that further investigation of this system could identify how dexamethasone-induced cataract occurs in humans.
Figure 2. Dexamethasone induces cataract-like effects in human micro-lenses after 8 days of treatment. (a) Phase contrast images show that exposing micro-lenses to increasing concentrations of dexamethasone (dex.) decreases light transmittance (top row) and focusing ability (bottom row). (b) Quantitative image analysis showing that increasing dexamethasone concentration significantly decreases micro-lens light transmittance compared to control (vehicle-only) treatment. (c) Quantitative image analysis showing that increasing dexamethasone treatment decreases micro-lens focusing ability compared to control (vehicle-only) treatment. The micro-lenses were derived from human-induced pluripotent stem cells, and were cultured for 10 days until light focusing occurred, after which time, treatment was initiated. Error bars represent standard error of the mean; eight micro-lenses from three independent experiments were analysed for each treatment.

9. Drug Toxicity Assessment Using ROR1-Expressing Lens Cells and Micro-Lenses

In addition to their potential for defining initiating mechanisms of human cataract formation, ROR1-expressing LECs and micro-lenses have significant potential for providing a new, large-scale and high-throughput system for assessing lens toxicity assessment. For example, new drugs could be tested using the micro-lenses to quantify their effects on human micro-lens transparency and/or focusing. Such an application would be consistent with how human iPS-cell-derived cardiomyocytes have been approved by the US Food and Drug Administration for cardiotoxicity assessment of new drugs [87]. Alternatively, older drugs that failed pre-clinical development due to the appearance of cataracts in animal models could be re-investigated using the micro-lens system—in order to determine whether they similarly cause cataract in functional human lens tissue.

Defining drug-induced molecular consequences within human micro-lenses could also identify potential cataract biomarkers to stratify patients at low- vs. high-risk of cataract formation. For example, higher concentrations of particular drugs may cause cataract formation [17]. Therefore, identifying patients that experience higher ocular drug concentrations (e.g., by quantifying drug concentration in the tear film) may enable stratification of patients into low- vs. high-risk of cataract formation. In turn, this information could identify patients in need of more frequent assessment of lens/eye health, and/or enable improved drug prescribing to minimize cataract formation in patients.

Defining molecular mechanisms of cataract formation using micro-lenses could also potentially identify candidate co-therapies to avoid cataract formation—in a similar way to co-therapies being used to avoid side-effects of other drugs. For example, folate is co-prescribed with methotrexate for rheumatoid arthritis/rheumatic diseases, in order to avoid hepatotoxicity and gastrointestinal side-effects caused by methotrexate-based treatment [88]. Defining the molecular mechanisms of
drug-induced cataract formation could offer similar opportunities for co-therapy development to avoid cataract formation.

10. Conclusions and Future Perspectives

Human PS-cell-derived lens cells and micro-lenses can provide a large-scale source of functional human lens tissue, possessing a range of morphological, molecular and functional similarities to primary human lenses. These ROR1-expressing lens cells and micro-lenses can be used to model individual or combined cataract risk factors, and associated human cataract initiation events in vitro. They can also be applied to small-scale or large-scale drug discovery and toxicity assays. Given the large annual financial burden cataract surgery places on health systems worldwide, investigating human cataract formation using ROR1-expressing lens cells and micro-lenses has significant potential to reduce the personal, social and economic consequences of cataract.

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