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Dermatological Health

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Table of Contents

Dermatological Health, 2024, Volume 2, Issue 3
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Clinical Efficacy of Transconjunctival Lower Eyelid Blepharoplasty for Treating Lower Eye Bags

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Abstract: *Objective*: To analyze the clinical effect of transconjunctival lower eyelid blepharoplasty for the treatment of lower eye bags. *Methods*: Retrospective analysis was done on 50 cases of patients with lower eye bags admitted to the hospital from August 2022 to August 2023. The control group received transcutaneous lower eyelid blepharoplasty, while the observation group received transconjunctival lower eyelid blepharoplasty. The efficacy, surgical indexes, and the difference in complications of the two groups were compared. *Results*: The efficacy of the observation group was higher than that of the control group ($P < 0.05$); the surgical time and postoperative skin recovery time of the observation group were shorter than that of the control group ($P < 0.05$); the rate of postoperative complications after blepharoplasty in the observation group was lower than that in the control group ($P < 0.05$). *Conclusion*: Transconjunctival lower eyelid blepharoplasty for the treatment of lower eye bags is safe and effective, which is favorable to the recovery of patients' skin appearance.

Keywords: Lower eye bags; Transconjunctival lower eyelid blepharoplasty; Clinical efficacy

Online publication: October 15, 2024

1. Introduction

Aging is a natural phenomenon that is related to metabolism and organ function decline. As a person ages, their facial skin gradually relaxes and wrinkles around the eyes gradually increase. The effects of orbital skin elasticity and periocular muscle relaxation will cause bags under the eyes to gradually appear in the middle-aged and elderly populations, which are manifested as lower eyelid sagging and fat accumulation [1]. Modern people are more concerned about facial aesthetics, so oculoplastic surgery has emerged as an industry. During plastic surgery treatment, cosmetic surgeons combine the patient's eye bag type to select the incision access program and orbital fat treatment program, which can assist the patient in restoring facial aesthetics [2]. At present, the commonly used clinical approach includes transconjunctival lower eyelid blepharoplasty, transcutaneous lower eyelid blepharoplasty, and other forms. Selective resection of orbital lipids during the operation can prevent the risks of postoperative scarring and lower eyelid ectropion. This paper analyzes the effect of transconjunctival lower eyelid blepharoplasty on 50 patients with lower eyelid bags.

2. General information and methods

2.1. General information

The data of 50 cases of patients with lower eye bags admitted to the hospital from August 2022 to August 2023 were analyzed retrospectively. The control group consisted of 25 cases with 3 men and 22 women, aged 36–46 years old and has an average age of 39.57 ± 1.49. The observation group consisted of 25 cases with 4 men and 21 women, aged 36–47 years old and has an average age of 39.61 ± 1.52. The inclusion criteria included first-time oculoplastic surgery for the patients; signed informed consent for plastic surgery; approval by the ethics committee; and indications for oculoplastic surgery. The exclusion criteria were contraindications to plastic surgery; history of lower eyelid surgery; ocular diseases; and severe vision loss. The data of patients with lower eye bags in the observation group were comparable with the control group, $P > 0.05$.

2.2. Treatment methods

Transcutaneous lower eyelid blepharoplasty was adopted in the control group. The medical staff instructed the patients to lie down in a flat position, then disinfected the face, laid a towel, and prepared local anesthesia with a concentration of 2% lidocaine (Kunming Jidai Pharmaceutical Co., Ltd.). The medical staff observed the onset of anesthesia and made a curved incision at the lower edge of the lower eyelid eyelashes with a length of 1–2 mm. The incision was extended at the vertical outer canthus to the area 3–8 mm outside the inner canthus, bluntly separating the orbicularis oculi muscle and the skin of the eye based on the line of the incision to expose the fat and orbital septal fascia thoroughly. Afterward, the medical staff excised the fat globules in the internal, intermediate, and external areas, and then ligatured the hemostasis after completion. The patient was guided to turn the eyelid upwards to reset the orbital septal fascia, and at the same time, the orbicularis oris muscle was lifted and fixed in the area of the outer canthus periosteum. Then, the orbital septal fascia was tightened by using absorbent threads, the skin of the incision area was sutured in place and the incision was closed. After plastic surgery, the incision was prepared by applying Dupix ophthalmic ointment (S.A. Alcon Couvreur N.V.) overlay, and the patients were instructed to avoid water until the stitches were removed. The medication was changed one day after the surgery and the stitches were removed five days postoperatively.

Transconjunctival lower eyelid blepharoplasty was performed in the observation group. The patients were instructed to lie down in a flat position for plastic surgery, face was disinfected, towels were spread, and 1 ml of lidocaine (Kunming Jidai Pharmaceutical Co., Ltd.) at a concentration of 2% was prepared for local anesthesia of the conjunctiva of the fornix of the lower eyelid. The medical staff observed the onset of anesthesia, turned down the patient’s lower eyelid until after full exposure of the lower eyelid plate, then made a transverse incision in the middle of the conjunctiva and eyelid fissure at the edge of the facial plate with a length of 1 mm. As the eyelid slit pulling hook traction line was pulling open the edge of the incision, ophthalmic scissors were used to gradually open the subconjunctival layer, bluntly separating and processing the subconjunctival orbicularis oculi muscle. After the full exposure of the orbital septum, the orbital septum was cut open, then gently pressing the eyeballs, separating the local puffed orbital fat mass. Excess fat was removed from the perichondrium with an electric cutter and the lower eyelid was gently lifted. Then the remaining fat was reset to the orbit, the incision was evaluated, the bleeding was stopped, and the incision was closed. After the plastic surgery, the patient applied eye ointment (S.A. Alcon Couvreur N.V.) to the incision in a covering type and was instructed to avoid water before removing the stitches. The medication is changed one day after the surgery and the stitches are removed five days after the surgery.

2.3. Observation indexes

The observation indexes included efficacy, surgical indicators, and adverse reactions. The efficacy indexes were as follows: significantly effective: the lower eyelid bloating and the ptosis disappeared, no discomfort, and the ratio of the lower eyelid bag groove height to the width of the eyelid fissure was reduced by more than 90%; effective: the lower eyelid bloating and ptosis improved, the eye was mildly uncomfortable when moving the eye, the lower eyelid bag ratio was reduced by 30–90%; ineffective: the lower eyelid bloating and ptosis was severe, the eye was seriously uncomfortable, and the lower eyelid bag ratio was reduced by less than 30%.

Surgical indicators included the time of surgery and postoperative skin recovery time of patients. Adverse reactions included patients' eye redness, swelling, incision infection, lower eyelid ectropion, pigmentation, and foreign body sensation.

2.4. Statistical methods

Data were processed using SPSS21.0 software, the χ^2 test was performed on the % count index, and the *t*-test was performed on the mean ± standard deviation (SD) measurement index. $P < 0.05$ indicated statistical differences.

3. Results

3.1. Comparison of plastic surgery efficacy

The efficacy of plastic surgery in the observation group was higher than that in the control group, with $P < 0.05$, as shown in **Table 1**.

Table 1. Plastic surgery efficacy [*n* (%)]

Groups	Significantly effective	Effective	Ineffective	Overall effective rate
Observation group (n = 25)	17 (68.00)	7 (28.00)	1 (4.00)	24 (96.00)
Control group (n = 25)	11 (44.00)	8 (32.00)	6 (24.00)	19 (76.00)
χ^2	-	-	-	4.153
P	-	-	-	0.042

3.2. Comparison of plastic surgery surgical indexes

The operation time and postoperative skin recovery time of the observation group were shorter than that of the control group ($P < 0.05$), as shown in **Table 2**.

Table 2. Plastic surgery surgical indexes (mean ± SD, points)

Groups	Surgical time	Postoperative skin recovery time
Observation group (n = 25)	1.98 ± 0.48	27.84 ± 1.25
Control group (n = 25)	2.79 ± 0.57	36.11 ± 1.48
t	5.435	21.345
p	0.000	0.000

3.3. Comparison of complication indicators

The complication rate after plastic surgery in the observation group was lower than that in the control group ($P < 0.05$), as shown in **Table 3**.

Table 3. Plastic surgery complications [n (%)]

Groups	Redness and swelling of the eyes	Infection of the incision	Lower lid ectropion	Pigmentation	Foreign body sensation	Total incidence
Observation group (n = 25)	0 (0.00)	0 (0.00)	1 (4.00)	0 (0.00)	0 (0.00)	1 (4.00)
Control group (n = 25)	1 (4.00)	1 (4.00)	2 (8.00)	1 (4.00)	1 (4.00)	6 (24.00)
χ^2	-	-	-	-	-	4.153
P	-	-	-	-	-	0.042

4. Discussion

Eye bags have symmetrical distribution characteristics, including the orbicularis oculi muscle laxity, fat volume and lower eyelid support structure imbalance, poor orbital septum support, and so on, which can lead to bulging and bloating of lower eyelid tissue after orbital fat displacement [3]. Middle-aged and elderly people have bags under their eyes due to aging and orbital septal fascia degeneration, while young people have bags under their eyes due to heredity conditions, sleep deprivation, and so on [4]. Eye bags have a great impact on human facial aesthetics, and patients are prone to a combination of keratitis, entropion, and other diseases. Mild eye bags generally can be relieved through the use of eye creams and regular rest, while moderate and severe bags require cosmetic surgery for orbital septum fat removal to restore the smoothness of the eyes [5]. There are many types of clinical plastic surgery, of which the most reasonable procedure should be chosen based on the characteristics of patients. At present, the common lower eye bag plastic surgery is mainly divided into two types: transconjunctival and transcutaneous approaches, which can restore facial aesthetics, but the effects of the surgical treatment types still need further in-depth research [6].

The transcutaneous approach has a high application rate, which can effectively remove the lower eye bags by making an incision through the lower eyelash margin, removing the bulging orbital septum, and disconnecting the excess skin, simultaneously treating the fat, orbital septum, orbicularis oculi muscle, and skin. Analyzing the treatment principle of the transcutaneous approach, the lower eyelid soft tissue tension is low, the skin, orbicularis oculi muscle, and orbital septum tissue are severely loose, and a large amount of fat accumulates behind the orbital septum. After the transcutaneous approach incision, the fat also automatically overflows. The removal of the excess fat globules can reduce the symptoms of sagging and laxity, so it is suitable for the treatment of patients with combined orbital fat prolapse of the lower eyelid bags [7]. The indications for the transcutaneous approach are broad, but there are some limitations. Transcutaneous approach surgery requires physicians' high experience and operating skills, and high precision in the selection of approach incisions, otherwise it can lead to large incisions and other adverse events. The transcutaneous approach has more bleeding and is prone to scarring, which may lead to medical disputes. This approach also has a long recovery cycle and a high risk of postoperative infection. In addition, the long postoperative recovery time may cause patients to develop negative emotions and reduce their quality of life.

The transconjunctival approach refers to making an incision at the conjunctiva of the lower eyelid and selectively resecting orbital septal fat based on the condition of patients with lower eyelid bags, which can reduce external tissue damage and the risk of scar exposure, so it has the advantages of shorter recovery time, less bleeding, and lower risk of postoperative incomplete closure and lower eyelid ectropion. Analyzing the principle of the transconjunctival treatment, patients with lower eye bags have loose orbital septum, sagging skin, and eye bags. The transconjunctival approach can reach the inner orbital septum and selectively resect orbital septal fat, which can improve the eye bags and reduce postoperative adverse reactions [8]. The advantages of the transconjunctival approach are summarized as follows. Small incisions can reduce the adjacent tissue damage, and almost no scar remains after surgery. There is no incision or scar on the lower eyelid, with a low risk of lower eyelid ectropion after surgery and little impact on their daily lives. The transconjunctival approach can accurately remove fat, and the small incision is suitable for later suturing, so the surgical risk is low.

According to the data analyzed in this article, the plastic surgery efficacy of the observation group was higher than that of the control group, $P < 0.05$. This suggests that the transconjunctival approach to plastic surgery has a better efficacy. Analyzing the reasons, the transcutaneous approach incision is large after tightening the orbicularis oris muscle resection, so it cannot completely reduce the orbicularis oris muscle laxity. For the transconjunctival approach to the anterior orbital septum operation, the lower eye bag excess fat removal can initially restore facial aesthetics. In addition, when the transconjunctival approach deals with the inner side, the orbicularis oris muscle is loosened at the starting point of the orbicularis oculi, the orbital part of the orbicularis oculi, and the tear trough ligament area, which can make this gap connect to the premaxillary space; when the outer side is dealt with, the orbicularis oculi muscle is loosened in the area of the supportive ligament and is continually extended and stripped to the zygomatic bone area, and at the same time, the residual fat is reset, which can improve the problem of eye bags effectively. Another set of data showed that the operation time and postoperative skin recovery time of the observation group were shorter than that of the control group, $P < 0.05$. This suggests that the transconjunctival approach can shorten the recovery time of patients with lower eye bags. The reason for this is that the transconjunctival approach to remove the fat from the bags under the eyes is less damaging to the ocular tissues, so the postoperative recovery is faster. In addition, the transconjunctival approach has a small operating range and will not adversely affect the patient's vision, which is safer. The last set of data shows that the postoperative complication rate of the observation group is lower than that of the control group, with $P < 0.05$, suggesting that the transconjunctival approach is safer. The reason for this is that in the transcutaneous approach, the amount of orbital fat removed is too much, and the surgical trauma is large, so it is easier to cause postoperative complications; while the transconjunctival approach makes a 1 mm incision in the middle of the conjunctiva and eyelid fissure at the edge of the face plate, which is less traumatic. After the selective removal of the fat with the electrosurgical knife, the remaining fat will be reset to the orbit, which reduces the degree of local damage and makes the operation safer. In addition, based on the analysis of the physiological anatomy of the eye, orbital fat refers to the adipose tissue in the orbital space, which has the function of protecting the optic nerve, fixing the eyeball, protecting the blood vessels, and cushioning the pressure of external force on the eyeball, and so on. After the accumulation of excessive orbital fat and the emergence of bags under the eyes, the conventional treatment via the skin approach involves the direct removal of the inflated fat and skin tissues, and the rest of the orbital fat has not been reset to its normal physiological structure. Excessive loss of orbital fat can lead to subsidence of the inferior rim, increasing the fine lines of the eyes, and then affecting the length and height of the tear trough, impairing facial aesthetics. Transconjunctival approach blepharoplasty is a small incision procedure that uses a blepharoplasty hook retractor to open the edge of the incision and the conjunctiva, detach the orbicularis oculi muscle, excise the excess fat, and reset the remaining fat to the orbit, which restores fixation of the orbital fat, dampens and protects the orbital fat, thus avoids the undesirable events of excessive orbital resetting.

5. Conclusion

In conclusion, transconjunctival lower eyelid blepharoplasty for the treatment of lower eye bags is popular due to its low postoperative complications and short postoperative skin recovery time.

Disclosure statement

The author declares no conflict of interest.

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Nanocellulose as Sustainable Eco-friendly Nanomaterials: Production, Characterization, and Applications

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Abstract: Nanocellulose is a biodegradable nanomaterial derived from lignocellulosic biomass through mechanical, chemical, or enzymatic defibrillation processes to convert wood fibers into nanofibrils. This material consists of a network of cellulosic fibrils with a wide range of diameters, offering unique properties suitable for various functional applications. Nanocellulose can act as a substrate or coating, serving as an eco-friendly alternative to synthetic plastics. Despite having good barrier properties and strong mechanical strength, nanocellulose films are still not as effective as synthetic plastics. They are used in creating barrier materials, flexible electronics, and coatings for paper products.

Keywords: Nanocellulose; Nanocomposites; Applications; Oxygen permeability; Water permeability

Online publication: September 30, 2024

1. Introduction

Packaging materials are essential components used to protect, contain, store, and transport goods and products. They come in various forms such as boxes, bags, containers, bubble wrap, tape, and labels. The choice of packaging materials depends on the product type, size, shape, weight, and fragility. Packaging materials serve several purposes, including protection, preservation, branding, sustainability, and convenience. Businesses must consider factors like cost, functionality, durability, and environmental impact when selecting packaging materials. Synthetic plastics are widely used as packaging materials due to their versatility, durability, and cost-effectiveness. They provide a strong barrier against moisture, oxygen, and light, extending the shelf life of products. However, the environmental impact of synthetic plastics, such as non-biodegradability and pollution, has raised concerns. Biodegradable plastics, compostable materials, and recyclable plastics are being developed to address these issues. The urgent need for sustainable alternatives to reduce negative consequences on the planet is emphasized. Plastics as packaging materials have significant disadvantages, including environmental pollution, non-biodegradability, microplastic contamination, resource depletion, toxic additives, greenhouse gas emissions, and single-use culture. Biopolymers, derived from renewable sources, offer a promising alternative to conventional plastics. They are renewable, biodegradable, have reduced environmental impact, are versatile, compatible with recycling, offer functional properties, and appeal to environmentally conscious consumers. Regulatory support further promotes the adoption of biopolymers in packaging. Biopolymers are gaining attention as sustainable alternatives to traditional plastic packaging materials due to their biodegradability, renewability, and reduced environmental impact. Polysaccharides and proteins are commonly used biopolymers for packaging applications, offering good barrier properties. However, challenges remain in scaling up production, cost-effectiveness, and performance under various storage conditions. Ongoing research aims to optimize the use of biopolymers in packaging materials. Papers and paper boards are widely used as packaging materials due to their versatility, sustainability, and cost-effectiveness. They are biodegradable, recyclable, renewable, and customizable, enhancing branding and product presentation. Factors like weight, fragility, and sensitivity to environmental factors should be considered when choosing papers or paper boards for packaging. Cellulosic fiber products like paper and paperboard offer eco-friendly properties and cater to diverse packaging needs. Cellulose fibers are environmentally friendly as they are biodegradable. In packaging, cellulose fiber products can be recycled but have limitations in water vapor and oxygen barrier performance. Cellulose nanofibers, like nanocellulose, offer enhanced strength and barrier performance, making them a sustainable material for various uses. Nanocellulose is sourced from renewable plant-based materials and can be tailored for specific applications. Therefore, the packaging industry is evolving towards more sustainable alternatives to reduce environmental impact and address plastic pollution. Biopolymers and cellulose-based materials offer promising solutions by providing eco-friendly, biodegradable, and renewable options for packaging materials. Ongoing research and development efforts aim to enhance the performance and usability of these materials, paving the way for a more sustainable packaging industry [1]. Cellulose is a versatile and sustainable biopolymer with strong mechanical properties and rigidity. It is biodegradable, recyclable, and sourced from plants like wood and agricultural waste. Cellulose offers solutions to reducing reliance on oil, combating plastic pollution, and lowering carbon footprints. It can be used in packaging applications to create biodegradable, compostable, and renewable materials that reduce plastic waste. Cellulose packaging also provides good barrier properties against oxygen and moisture, extends shelf life, and reduces transportation costs and carbon emissions. Challenges like cost and processing techniques need to be addressed to optimize cellulose use in packaging. Additionally, cellulose is used in various industries for its mechanical properties and barrier performance. Nanocellulose, derived from plant fibers, offers advantages in sustainable packaging such as excellent barrier properties, high strength, biodegradability, versatility, and compatibility with existing processes. Overall, nanocellulose shows great promise as a green alternative to traditional packaging materials, meeting environmental demands for sustainable solutions and high-performance packaging [2-5]. Several questions remain: What are the key advantages of using cellulose in packaging applications? What challenges need to be addressed to optimize the use of cellulose as packaging materials? How does nanocellulose differ from traditional packaging materials in terms of sustainability and performance?

Cellulose is a straight-chain carbohydrate polymer made up of repeating units of cellobiose, a disaccharide composed of two β-glucose molecules connected by a β(1→4) bond. Being a natural polysaccharide, it is prone to degradation by microbes and fungi. In woods, cellulose is present as an arrangement of cellulose fibril chains that form a well-organized fiber wall. **Figure 1** illustrates the organization of cellulose fibers from wood to a single fiber, while **Figure 2** depicts the structure of cellulose and its surface hydroxyl groups [6]. **Figure 3** displays cellulose with two distinct domains: crystalline and amorphous regions [7].

The naming conventions for cellulosic nanomaterials have not been consistently reported in the literature. According to the TAPPI Standard WI 3021, various terms are used to describe these materials, such as cellulose nanomaterial, cellulose nano-object, cellulose nanostructured material, cellulose nanofiber, cellulose nanocrystal, and cellulose nanofibril. The diameter of cellulose fibers is reduced through mechanical, chemical, and enzymatic processes to create nanocellulose. Micro-fibrillated cellulose (MFC) is produced by delaminating wood fibers using mechanical pressure along with chemical or enzymatic treatment. MFC is synonymous with nanofibrils, microfibrils, and nanofibrillated cellulose, with diameters ranging from 10 nm to 100 nm, fibril lengths between 500 and 10,000, and an aspect ratio of 100. MFC is primarily made up of cellulose semi-microcrystalline fibrils generated through high-pressure homogenization of wood pulp [8-11].

Nanocellulose offers several advantages as a barrier material: (1) High mechanical strength: Nanocellulose has excellent mechanical properties, providing a strong barrier against external elements. (2) Low permeability: Its dense structure and high surface area make nanocellulose an effective barrier against gases, liquids, and other substances. (3) Renewable and sustainable: Nanocellulose is derived from natural sources like wood pulp and plants, making it an eco-friendly alternative to synthetic barrier materials. (4) Biodegradable: Due to its natural origin, nanocellulose is biodegradable and does not contribute to environmental pollution. (5) Versatility: Nanocellulose can be modified to suit different applications and requirements, making it a versatile choice for a range of barrier materials. (6) Transparent: In certain forms, nanocellulose can be transparent, making it suitable for applications where visibility is important. (7) Chemical resistance: Nanocellulose exhibits good chemical resistance, providing protection against corrosive substances. (8) Thermal stability: Nanocellulose can offer thermal insulation and stability, making it suitable for applications where temperature control is critical.

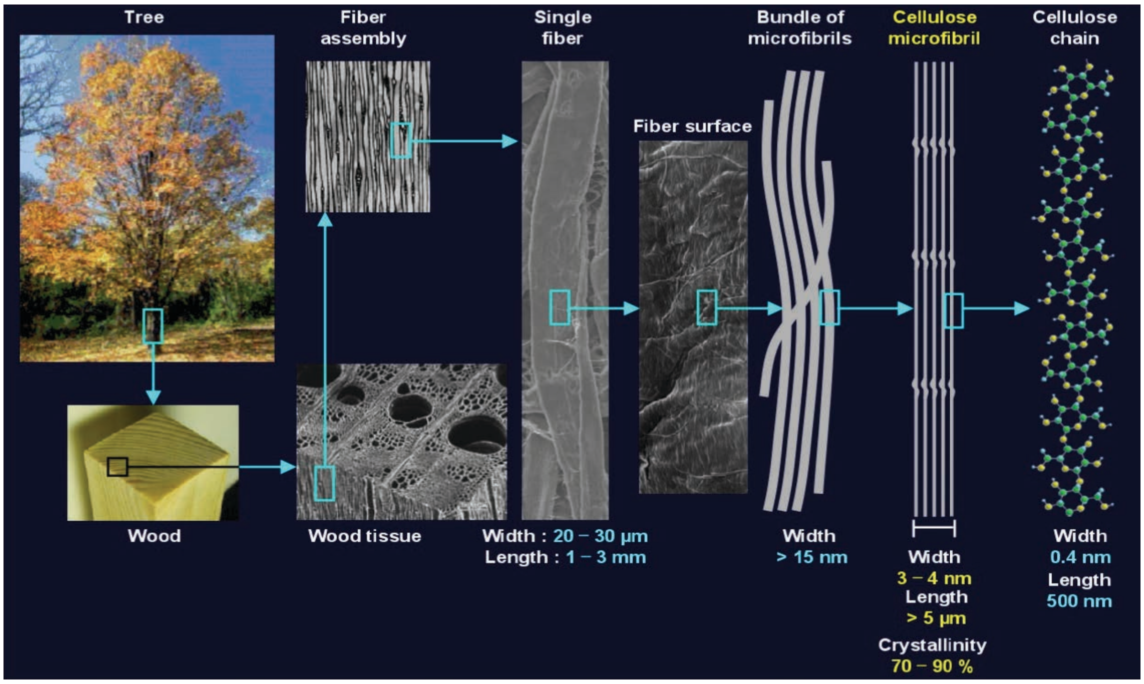

Figure 1. Hierarchical structure of cellulose from wood to the molecular level cellulose [1]

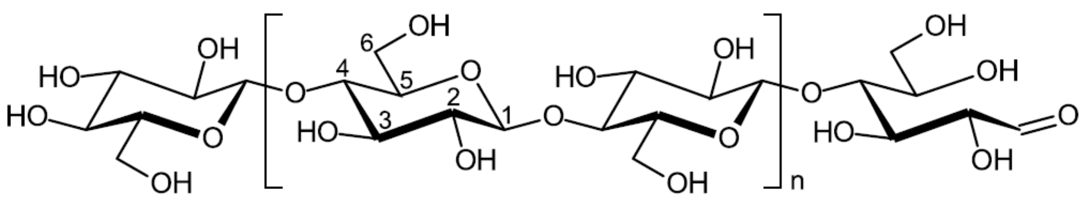

Figure 2. Molecular structure of cellulose [6]

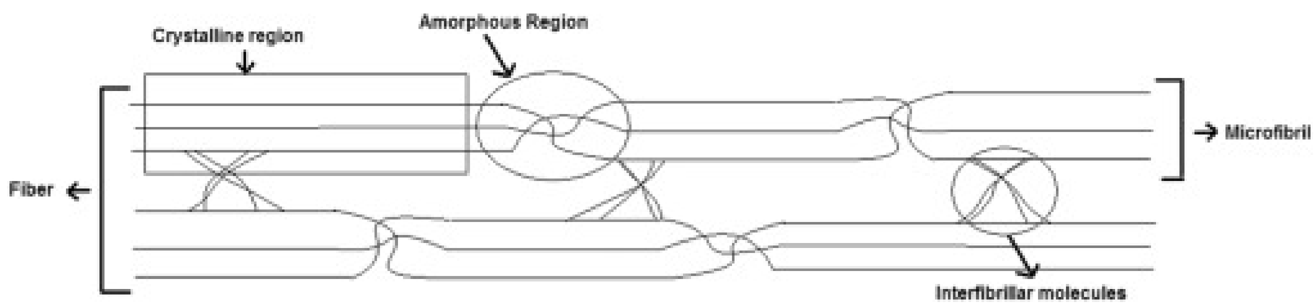

Figure 3. Crystalline and amorphous regions of cellulose [7]

2. Nanocellulose as a novel fibrous material

Nanocellulose is a novel fibrous material derived from cellulose fibers that have been broken down to the nanoscale level, typically through mechanical or chemical processes. This material exhibits exceptional strength, stiffness, and biodegradability, making it a promising alternative to traditional materials in various industries such as automotive, aerospace, packaging, and biomedical. Due to its unique properties, nanocellulose has gained attention for applications in advanced composites, films, coatings, and even as a reinforcement in plastics and paper products. Its renewable and sustainable nature further enhances its appeal as an eco-friendly material that can help reduce the environmental impact of industries. Nanocellulose's versatility and potential for innovation have sparked research and development efforts to explore new applications and manufacturing techniques. As a fibrous material with nanoscale dimensions, nanocellulose offers a wide range of possibilities for creating high-performance and environmentally friendly products with enhanced mechanical properties and functionalities. Overall, nanocellulose represents a promising avenue for the development of advanced materials that can meet the demands of a sustainable and resource-efficient future.

Nanocellulose (NC) can be categorized into three types: cellulose nanocrystals (CNC), cellulose nanofibrils (CNF), and bacterial cellulose (BC). This study focuses on the use of cellulose nanofibers, which form a tangled network of micro and nanofibrils containing both amorphous and crystalline regions. These regions play a key role in the functionality of materials made from NC. The nanofibrils have a high aspect ratio, with a diameter of approximately 20 nm after undergoing high-pressure homogenization. NC exhibits a high elastic modulus (150 GPa) and tensile strength (10 GPa), with these properties being influenced by the diameter and length of the cellulose fibrils. The aspect ratio of the fibrils is determined by factors such as the source, processing method, and particle type. The properties and applications of NC are also influenced by its source, affecting its functionality in different fields [11-14]. Nanocellulose consists of cellulose fibrils with varying diameters and lengths, typically several micrometers long. These fibrils contain both crystalline and amorphous regions, with the crystalline portion playing a significant role in the material's functionality, particularly in barrier and composite materials. The diameter distribution and aspect ratio of nanocellulose are influenced by factors like the source, pre-treatment processing, and fibrillation method, which in turn affect its rheological and interfacial properties [15,16]. NC under a scanning electron microscope is shown in **Figure 4**.

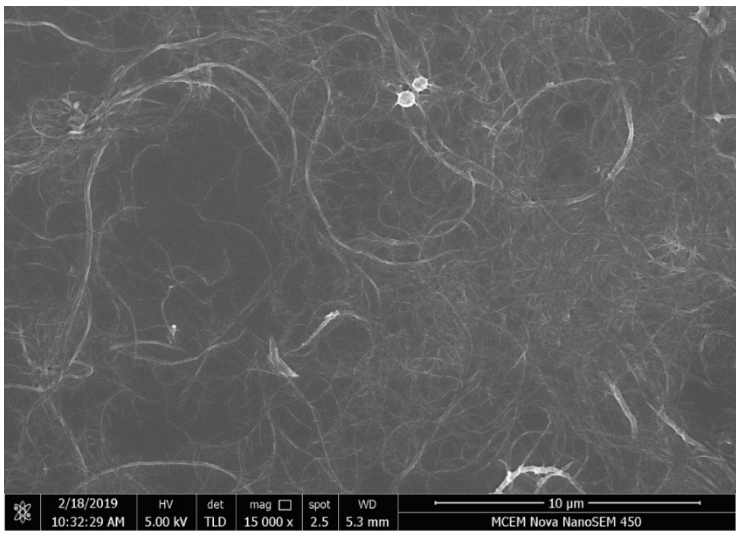

Figure 4. SEM micrograph of nanocellulose (CELISH KY-100S treated with 2-pass high-pressure homogenization)

2.1. Characteristics of nanocellulose

Nanocellulose is a versatile nanomaterial derived from cellulose fibers, possessing unique properties that make it suitable for various applications. Some key characteristics of nanocellulose include: (1) High strength: Nanocellulose has exceptional mechanical properties, such as high tensile strength and stiffness, making it stronger than steel on a weight-to-weight basis. (2) Lightweight: Nanocellulose is light in weight, which is advantageous for applications where weight reduction is important. (3) Renewable: Nanocellulose is derived from renewable sources such as wood pulp or agricultural waste, making it an eco-friendly and sustainable material. (4) High surface area: Nanocellulose has a high surface area due to its nano-sized dimensions, providing opportunities for functionalization and enhancing its interactions with other materials. (5) Biodegradable: Nanocellulose is biodegradable and environmentally friendly, making it a desirable alternative to petroleum-based materials. (6) Transparent: Nanocellulose films can be transparent, offering possibilities for applications in optoelectronics and packaging. Overall, the unique combination of high strength, lightweight nature, renewability, biodegradability, high surface area, and transparency makes nanocellulose a promising material for a wide range of industries, including composites, food packaging, biomedical applications, and electronics.

The morphology of nanocellulose refers to its structure and arrangement at the nanoscale level. Nanocellulose materials can exhibit diverse morphologies depending on factors such as the source of cellulose, processing methods, and post-treatment techniques. Below are some common morphologies of nanocellulose:

2.1.1. Cellulose nanocrystals (CNCs)

(1) Rod-like shape: CNCs typically have a rod-like or needle-like morphology with high aspect ratios. They

are obtained through the controlled hydrolysis or enzymatic treatment of cellulose fibers, resulting in the removal of amorphous regions and the isolation of crystalline segments.

(2) Uniform size: CNCs usually have a uniform size distribution, with diameters typically in the range of a few nanometers to tens of nanometers and lengths ranging from a few hundred nanometers to several micrometers.

2.1.2. Cellulose nanofibrils (CNFs)

(1) Fibrillar structure: CNFs consist of interconnected fibrillar structures with diameters typically in the nanometer range. They are obtained through mechanical disintegration or enzymatic treatment of cellulose fibers, leading to the separation of individual fibrils.

(2) High aspect ratio: CNFs can have high aspect ratios, similar to CNCs, but with a more entangled and less ordered structure compared to CNCs.

2.1.3. Bacterial cellulose (BC)

(1) Highly pure and crystalline: BC is produced by certain bacterial strains, such as *Acetobacter xylinum*, through the biosynthesis of cellulose. It has a highly pure and crystalline structure, with nano-sized fibrils arranged in a highly organized manner.

(2) Nanostructured network: BC forms a three-dimensional network structure of nanofibers, offering unique properties such as high mechanical strength, high water-holding capacity, and biocompatibility.

2.1.4. Tunicate cellulose

Nanofibrillar network: Tunicate cellulose, obtained from sea squirts or tunicates, consists of a nanofibrillar network with high aspect ratios and uniform morphology. It is known for its exceptional mechanical properties and purity.

2.1.5. Whisker-like structures

Some nanocellulose materials may exhibit whisker-like structures, which are elongated nanostructures with tapered ends. These structures can be found in various forms of nanocellulose, such as CNCs and certain types of CNFs.

Overall, the morphology of nanocellulose materials plays a critical role in determining their properties and potential applications. Understanding and controlling the morphology of nanocellulose is essential for the development of tailored nanocellulose-based materials with desired properties for various industrial and biomedical applications.

The size and shape of NC can be determined by analyzing images obtained from scanning electron microscopy, transmission electron microscopy, and atomic force microscopy. Additionally, the gel point of NC fibers can be assessed through sedimentation tests. The gel point is the point at which a network of fibers becomes mechanically stable under load at the lowest solid concentration. This marks the transition from a very dilute solution of NC fibers to a concentrated suspension, where nanofibrils form a dense network due to hydrogen bonding. This network, known as the connectivity threshold, is crucial for maintaining mechanical strength. Beyond this point, the suspension loses strength as fibers have insufficient contact. This property is influenced by the aspect ratio of the nanocellulose fibers and impacts the drainage time during vacuum filtration for NC film production [17,18].

Key characteristics of nanocellulose include its lightweight nature, transparency, chemical versatility, stability in size, and effective barrier properties. Nanocellulose shows promise for use in biomedical applications and composite development due to its potential compatibility with other materials such as natural polymers, proteins, and cells. The presence of surface hydroxyl groups on nanocellulose allows for chemical modifications and functionalization. The rheological properties of nanocellulose are crucial in the coating process for creating protective layers on paper substrates, exhibiting pseudoplastic behavior under shear-thinning conditions [19].

The main goal of this review is to thoroughly examine the production of NC and NC composite films, pinpoint research gaps, and explore ways to expedite the formation of NC films. The potential uses of nanocellulose films and composites, such as barriers, fire retardant layers, smooth films, membranes, and other applications, will also be explored. The review will also discuss the sustainability of nanocellulose as a barrier material and the need for enhancing its barrier performance against water vapor, particularly through the development of composites with nanoclay.

2.2. Nanocellulose production

Nanocellulose is typically produced through the mechanical or chemical treatment of cellulose fibers found in plants. Mechanical methods involve breaking down the cellulose fibers using high-pressure homogenizers or microfluidizers to produce nanocellulose with high aspect ratios. On the other hand, chemical methods such as acid hydrolysis or oxidation are used to dissolve the cellulose and then regenerate it in the form of nanocellulose. One commonly used method for nanocellulose production is the acid hydrolysis of cellulose with sulfuric acid, which breaks down the cellulose into nanofibrils or nanocrystals. This process requires precise control of reaction conditions such as temperature, acid concentration, and reaction time to obtain nanocellulose with desired properties. Another method involves the enzymatic hydrolysis of cellulose using cellulase enzymes, which can selectively break down cellulose fibers into nanocellulose without the need for harsh chemicals. This method is considered more environmentally friendly but may be slower and more expensive than acid hydrolysis. Overall, the production of nanocellulose is a promising field with numerous potential applications in various industries due to its renewable and biodegradable nature, high strength, and unique properties. The main sources of cellulose include wood, seed fibers (such as cotton, and coir), bast fibers (like flax, hemp, jute, and ramie), and grasses (such as bagasse, and bamboo). On the other hand, marine animals (tunicate), algae, fungi, invertebrates, and bacteria are the least common sources of cellulose. Wood is the primary source of cellulose and is categorized into hardwood and softwood. In addition to cellulose, wood also contains hemicellulose, lignin, and inorganic salts. The key distinction between hardwood and softwood lies in the structural complexity and level of heterogeneity in cellulose fibrils [20,21]. The cellulose fibers extracted from hardwood pulp were more difficult to convert into nanocellulose through fibrillation compared to those from softwood pulp. When hardwood pulp is subjected to high-pressure homogenization, it can lead to pressure fluctuations in the homogenizer and equipment clogging. Hardwood pulp is more prone to forming fiber aggregates during processing than softwood pulp, requiring a higher number of passes to break these aggregates. The structure of hardwood makes it resistant to the fibrillation process, with only the outer layer of the cell wall being fibrillated. Essentially, the surface of the fibers from hardwood was transformed into cellulose nanofibers through fibrillation [22]. Hardwood fibers are stiffer compared to softwood fibers due to a high Runkel ratio, which means they have a thick fiber wall in relation to their internal diameter. It has been noted that less energy is required to process softwood into NC compared to hardwood [22,23].

The text mentions that hardwood pulp from various trees like gum, maple, oak, eucalyptus, poplar, beech, or a combination of these is used for producing nanocellulose. Softwood pulp from northern spruce and Scots pine is also used for this purpose. When processing hardwood for making nanofibers, the number of passes in high-pressure homogenization increases, requiring a significant amount of energy for fibrillation. The amount of energy needed to produce nanocellulose from bleached eucalyptus kraft pulp using mechanical fibrillation ranges from 5 to 30 kWh/kg. Similarly, non-woody sources like grasses and marine materials require minimal energy to produce nanocellulose due to their low lignin content and cellulose structure that is well-suited for purification and fibrillation. Agricultural and food waste have also shown promise as viable sources for nanocellulose production through a chemical-free method [23,24].

Fibrillating microfibrils into nanofibrils through the refining process poses a significant challenge. Refining methods can be categorized as mechanical, chemical, or enzymatic. Prior to refining microfibrils, fibers undergo pre-treatment like delignification to decrease the energy needed for refining and creating high-quality nanofibers. Delignification involves separating lignin from lignocellulosic biomass, achieved through a pulping process that breaks down lignin and hemicellulose, which are then washed away. Additional delignification can be done through bleaching with chemicals. These pre-treatments can cause mechanical and chemical alterations to cellulose. They result in high-quality cellulose fibers by eliminating non-cellulosic components. The pre-treatments help break down cellulose fibrils, reducing fiber aggregation and preventing clogs during further fibrillation. This leads to improved fibrillation into nanofibers and reduced energy consumption [7].

2.2.1. Mechanical process

Nanocellulose is produced through mechanical processes by breaking down cellulose fibers into nano-sized particles. One common method is called high-pressure homogenization, where cellulose fibers are subjected to high pressure and shear forces to break them down into nanoscale dimensions. Another method is microfluidization, which involves passing cellulose suspensions through narrow channels at high velocities to produce nanocellulose. Mechanical processes for nanocellulose production offer advantages such as scalability, cost-effectiveness, and environmental friendliness compared to chemical processes. These methods typically do not require harsh chemicals or solvents, making them more sustainable and suitable for various applications including biomedical, materials, and environmental fields. Furthermore, nanocellulose produced by mechanical processes exhibits unique properties such as high strength, biodegradability, and biocompatibility, making it a promising material for various advanced applications. Overall, mechanical processes for nanocellulose production offer a promising avenue for sustainable manufacturing of high-performance nanocellulose materials with a wide range of applications.

The most common way to turn cellulose fibers into nanofibers mechanically is through disintegration, with grinding being a viable method for producing nanocellulose. During the grinding process, fibers are squeezed between a rotor and stator disc, leading to their disintegration due to frictional forces and the high impact of grinding. This method does not cause clogging and does not require any pre-processing of the fibers. Another method, high-intensity homogenization, has been introduced as a new way to produce nanofibers from cellulose macrofibers and particles. However, it is an energy-intensive process, requiring around 25,000 kWh per ton of microfibrillated cellulose production. These techniques can be scaled up for NC production. In high-pressure homogenization, factors such as pressure, NC solid concentration, and the number of passes play a crucial role in energy consumption. Various mechanical fiber disintegration methods are outlined in **Table 1**, summarizing the reduction of cellulosic fibers, isolation methods, as well as their advantages and disadvantages [5,10].

The research comparing energy usage in the production of microfibrillated cellulose found that using a homogenizer results in high specific area microfibrillated cellulose, leading to films with excellent barrier properties, including a low water vapor transmission rate of 3.81 x 10^{-4} mol/m^2.s. However, the homogenization process requires a significant amount of energy to produce the microfibrillated cellulose. This high energy consumption is a major obstacle in creating environmentally friendly packaging materials from these substances. The energy consumption order for producing MFC for film-making is high-pressure homogenization > micro fluidizer > grinder with fiber pre-treatment [25-27].

Table 1. Mechanical refining for the production of nanocellulose [10]

Methods for isolation of cellulosic fibers	Concept of reduction of size	Advantages	Disadvantages	References
High-pressure homogenization	High-impact shearing forces reduce fiber size	Quick, effective, and continuous process; Good reproducibility could control the degree of defibrillation	Clogging; Pre-treatment of fibers required; High passing time and high energy consumption; Rise in temperature of suspension.	[28] [10]
Micro fluidization	An intense collision with high impact led to the splitting of macrofibers into nanofibrils.	Less clogging; Uniformity in size; Lesser cycles	Not suitable for scale-up	[28]
Micro grinding	The macrofibers are pressed in the gap between the stator and rotor disc in the grinder. High impact and frictional forces disintegrate the fibers into fibrils.	Less energy and cycle; No pre-treatment of fibers	High cost; Difficult in replacement of internal parts such as disk; The crystalline nature of nanocellulose is reduced	[10]
High-Intensity ultrasonication	Sound energy is utilized to disintegrate macro fibers into nanofibrils.	High power output and high efficiency of defibrillation	Lab-scale application	[10]
Refining	High shearing forces are used for disintegration.	-	-	[10] [29]
Cryo-crushing	The refined macrofibers are treated with liquid nitrogen and it freezes the water in the fibers and then subjected to high-impact grinding for disintegration.	High disintegration performance	Ice formation	[30] [9] [10]
Steam explosion	The suspension is heated at high pressure and then vented into a vessel with low pressure. It is a type of explosion for the reduction of fibers.	-	Chemical pre-treatment required	[31] [10]

2.2.2. Chemical process

Nanocellulose, a renewable and sustainable nanomaterial, can be produced via chemical processes such as acid hydrolysis, oxidation, and esterification. Acid hydrolysis involves breaking down cellulose fibers into nanoscale dimensions using strong acids like sulfuric acid. This process results in cellulose nanocrystals (CNCs) or cellulose nanofibrils (CNFs) depending on the reaction conditions. Oxidation methods, such as TEMPO-mediated oxidation, involve the selective oxidation of cellulose to introduce carboxyl or aldehyde groups, facilitating the disintegration of cellulose into nanoscale dimensions. Esterification processes modify cellulose by introducing organic ester groups through reactions with acid anhydrides or acids, leading to the formation of nanocellulose derivatives with unique properties. These chemical processes allow for the production of nanocellulose with tailored properties like high aspect ratios, large surface areas, and excellent mechanical strength. Nanocellulose has a wide range of applications in industries such as packaging, biomedical, textiles, and composites due to its biodegradability, renewability, and compatibility with other materials. However, the choice of chemical process influences the properties and potential applications of the resulting nanocellulose materials.

Breaking down large fibers into smaller pieces requires a significant amount of energy, which can be a challenge when trying to increase production scale. The demand for cellulosic materials is growing rapidly in research and industry, highlighting the need for an energy-efficient production process for nanocellulose. Chemical or enzymatic methods are often more efficient in terms of energy consumption and can enhance the process of breaking down fibers into nanocellulose. Different chemical processing techniques for cellulosic nanofibers are discussed in **Table 2**, outlining their pros and cons [6,10].

Table 2. Chemical processing for nanocellulose [10]

Serial no.	Chemical methods	Reaction mechanism	Advantages	Disadvantages
1	TEMPO-oxidation	Oxidation of C6 hydroxyl groups into carboxyl groups and partially into aldehydes.	Shorter reaction time	TEMPO is a poisonous and expensive chemical reagent
2	Periodate chlorite oxidation	Oxidation of the vicinal hydroxyl groups in the C2 and C3 positions.	Increased carboxyl group	Weakens the structure of cellulose; Long reaction time
3.	Carboxymethylation	Incorporating carboxymethylated cellulose to the fibers. Enhancing anionic groups, reduction of fiber friction, disintegration into fibrils	Improved fibrillation; Reduced energy consumption	Thinner NFC produced

Table 2 outlines the reaction mechanisms involved in various chemical methods used to convert cellulose into nanocellulose. These methods are typically used as pre-treatments for cellulose fiber pulp suspensions derived from wood or other cellulose-based sources. One example is the production of nanocellulose through TEMPO

oxidation, followed by high pressure homogenization to further break down the cellulose into nanofibrils.

2.2.3. Enzymatic process

Nanocellulose production via an enzymatic process involves breaking down cellulose fibers into nano-sized particles using enzymes. This process typically starts with the extraction of cellulose from plant sources such as wood pulp or agricultural residues. The cellulose is then pretreated to remove impurities and make the fibers more accessible to enzymatic action. Enzymes like cellulase are then used to hydrolyze the cellulose chains into smaller units, ultimately yielding nanocellulose. The enzymatic process offers advantages such as high specificity, mild reaction conditions, and environmentally friendly production compared to traditional mechanical methods. The resulting nanocellulose has unique properties like high strength, biodegradability, and large surface area, making it a promising material for various applications in industries such as packaging, textiles, biomedical devices, and composites. Overall, the enzymatic production of nanocellulose presents a sustainable and efficient approach to creating high-performance materials with a wide range of practical uses. Enzymatic processes can be utilized to create nanocellulose from cellulosic biomass. Enzymatic hydrolysis involves breaking glycosidic bonds within cellulose fibers. Cellobiohydrolases and endoglucanases are the main enzymes involved, targeting different regions of cellulose. The efficiency of nanocellulose production through fibrillation depends on the duration of enzymatic treatment and enzyme concentration. Enzymatic hydrolysis offers benefits such as effective fibrillation, decreased fibril clogging in mechanical processes, and lower energy consumption during processing [10].

3. Application of nanocellulose

The small size of nanocellulose in terms of fiber diameter and length, along with its large surface area, presents a significant opportunity to create a more versatile material for a variety of uses. The demand for nanocellulose is on the rise due to its numerous technical applications. One notable application is in the production of nanocellulose film, which is becoming increasingly important. This film consists of transparent, densely packed cellulose nanofibrils that offer excellent barrier properties and smoother surfaces compared to paper. This has led to the development of various functional materials. Nanocellulose film can be utilized in high-performance packaging and serves as a suitable substrate for flexible and printable electronics, cost-effective diagnostics, and organic displays. While there are numerous applications for nanocellulose film, this chapter focuses on discussing a few key uses [32,33].

Free-standing nanocellulose films have various applications due to their unique properties such as high strength, flexibility, biodegradability, and sustainability. Some common applications include: (1) Packaging: Nanocellulose films can be used as sustainable and biodegradable packaging materials for food, pharmaceuticals, and other products. (2) Biomedical: These films can be used for wound healing, drug delivery systems, and tissue engineering due to their biocompatibility and ability to degrade in the body. (3) Sensors: Nanocellulose films can be used to develop flexible and biocompatible sensors for various applications such as healthcare monitoring and environmental sensing. (4) Membranes: They can be used as filtration membranes for water purification and gas separation due to their high mechanical strength and porosity. (5) Electronic devices: Nanocellulose films can be used in flexible electronics, such as displays, touch screens, and energy storage devices due to their mechanical flexibility and transparency. Overall, free-standing nanocellulose films have a wide range of applications across different industries due to their unique properties and environmentally friendly nature.

3.1. Barrier applications

Barrier materials are commonly used to protect foods, nutrients, drinks, pharmaceuticals, and cosmetics from physical, chemical, and microbiological deterioration. These materials need to have low gas and water permeability to shield the contents from external factors and maintain the quality and characteristics of the packaged product. Glass, metals like aluminum and tin, and synthetic plastics derived from fossil fuels are frequently employed as barrier materials due to their effective protective properties and durability. However, these materials are not environmentally friendly as they are not biodegradable or recyclable. Presently, packaging materials are expected to have minimal gas and water vapor permeability [34-36].

Currently, synthetic plastic films and sheets are widely used but are often discarded as waste in landfills, posing environmental threats. Recycling these plastics is costly and maintaining their barrier performance after recycling is challenging. Cellulosic fiber products like paper and paperboard are biodegradable alternatives for flexible packaging. However, the hydrophilic nature of cellulose limits its water vapor and oxygen barrier properties. This results in poor barrier performance due to large pore sizes and high water affinity. To address these limitations, paper and paperboard can be coated with plastics, wax, or extruded aluminum to enhance their barrier properties. Nevertheless, these composite materials are challenging to recycle [37]. Free-standing nanocellulose films find various applications as barriers due to their exceptional mechanical properties, high surface area, and renewable nature. When applied as a barrier, nanocellulose films can provide protection against gases, liquids, and even microorganisms. In packaging, nanocellulose films can be used to improve the barrier properties of materials, thus extending the shelf life of food products and reducing food waste. These films can also be utilized in the pharmaceutical industry to create drug delivery systems with controlled release properties. Moreover, they can be employed in electronics to provide protection against moisture and oxygen, enhancing the longevity and performance of electronic devices. In the medical field, nanocellulose films can act as barriers to prevent the passage of bacteria and other contaminants, making them useful in wound dressing materials and surgical implants. Overall, the exceptional barrier properties of free-standing nanocellulose films make them versatile materials with promising applications in various industries, contributing to the development of more sustainable and high-performance products.

Nanocellulose possesses effective barrier properties because of its fibrous network and crystalline region [13]. The cellulose nanofibrils within this dense network create a tight structure that limits the passage of water vapor and oxygen. This intricate fibrous network increases the pathway for oxygen and water vapor permeation, ultimately creating a barrier against these substances. Additionally, the barrier performance of nanocellulose film can be improved by increasing tortuosity through the inclusion of montmorillonite clay in the fibrous network. Nanocellulose film exhibits low oxygen permeability (OP) as a result of the complex interweaving of fibrils [37]. The permeability of the NC film is said to be 0.004 $cm^3.\mu m.m^{-2}.day^{-1}.kPa^{-1}$, which is similar to synthetic plastics like polyvinylidene (PVDC) with a permeability of 0.1–3 $cm^3.\mu m.m^{-2}.day^{-1}.kPa^{-1}$ and poly(vinyl chloride) (PVC) with a permeability of 20–80 $cm^3.\mu m.m^{-2}.day^{-1}.kPa^{-1}$ [38,39]. The ability of NC films to act as a water barrier is excellent in dry conditions, although it is not as effective in high humidity [26]. This makes NC a promising substitute for synthetic plastics in barrier applications [40]. The source of fibers, chemical composition, physical structure, and pretreatment techniques used in producing nanocellulose all influence its barrier properties [9].

3.2. Printed electronics

Cellulose-based materials like paper are cost-effective and sustainable substrates for creating functional materials such as printed electronics, solar cells, RFID tags, OLEDs, PV cells, printed diagnostics, batteries, memory cards, transistors, and supercapacitors. However, paper's porous nature, high susceptibility to moisture absorption, and rough surface texture limit its use in electronic material construction. The surface roughness of paper substrates typically ranges from 2 to 10 microns, which can hinder the performance of solar cells. To address this, nanocellulose is being explored as a superior substrate for printed electronics due to its smoother surface, thermal stability, and effective barrier properties against water and air, making it ideal for flexible electronic device fabrication [41-44].

3.3. Other applications

The characteristics and performance of nanofibrillated cellulose can be customized. Nanocellulose films can exhibit exceptional mechanical, optical, and structural features, which are beneficial for creating a range of functional materials like cellulose nanocomposites, inorganic nanocomposites, organic transistors, conducting materials, immunoassays, and diagnostic materials [45-49]. Therefore, nanocellulose is utilized in photonics, as a surface modifier, in nanocomposites, biomedical scaffolds, and optoelectronics [13]. Recently, nanocellulose films have emerged as a highly promising functional material with various applications such as virus removal filters, adsorbents, catalysts [50], cell culture substrates, thermal insulators, and drug carriers for drug delivery systems [51]. Additionally, nanocellulose's barrier properties and size make it suitable for functionalizing base sheets by creating barrier layers as coatings or producing freestanding sheets/films and nanocomposites [6]. The use of nanocellulose, which has been developed through academic research, has been growing rapidly. While there are currently no nanocellulose films being used commercially, there are products like TEMPO nanocellulose in Japan and Pure nanocellulose produced in Canada and the USA. Several companies, including CelluForce, Kruger, Performance Bio Filaments in Canada, VTT in Finland, and InoFib in France, are involved in the commercial production of nanocellulose. Nanocellulose is being utilized as a raw material for film development, with a need for a faster manufacturing process for nanocellulose films, especially for barrier applications to replace synthetic plastics. The text also discusses the various applications of nanocellulose in different fields and showcases products developed with nanocellulose (**Figure 5**) [52].

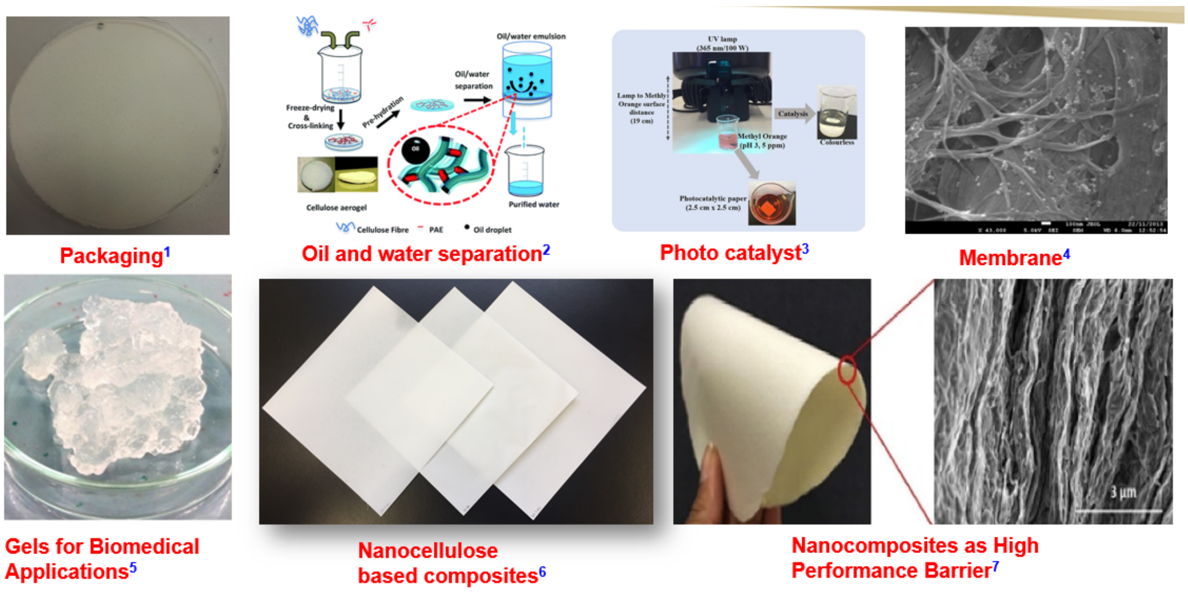

Figure 5. Applications of nanocellulose into functional materials from high-performance fiber material research group, BIOPRIA, Monash University, Australia.

4. Products from free-standing nanocellulose films

4.1. Packaging

A homogenized NC film was created by MFC using vacuum filtration, resulting in an air permeance of less than 0.003 μm/Pa.S, indicating its effectiveness as an impermeable film for packaging purposes. The NC film exhibited a water vapor transmission rate (WVTR) of 44.7 g/m^2.day and an oxygen transmission rate (OTR) of 20.1 cc/m^2.day at 23°C and 50% RH. These findings demonstrate that a vacuum-filtered NC film can serve as a reliable barrier [53,54]. Free-standing nanocellulose films have various potential packaging applications due to their unique properties. These films are lightweight, transparent, flexible, biodegradable, and have excellent barrier properties against oxygen and oils.

(1) Food packaging: Nanocellulose films can be used in food packaging to protect products from contamination, moisture, and oxygen exposure. These films can extend the shelf life of food products and maintain their freshness.

(2) Biomedical packaging: Nanocellulose films can be used in medical and pharmaceutical packaging due to their biocompatibility and barrier properties. They can help protect medical devices, implants, and pharmaceutical products from environmental factors.

(3) Sustainable packaging: Nanocellulose films are environmentally friendly and biodegradable, making them an attractive option for sustainable packaging solutions. They can help reduce reliance on traditional plastic packaging materials.

(4) Electronics packaging: Nanocellulose films can also be used in electronics packaging to provide protection against moisture and other contaminants. Their flexibility and barrier properties make them suitable for various electronic devices.

Therefore, free-standing nanocellulose films have the potential to revolutionize the packaging industry by offering sustainable, biodegradable, and high-performance packaging solutions for various applications.

4.2. Oil and water separation

Nanocellulose and polyamidoamine-epichlorohydrin (PAE) were crosslinked to create an aerogel filter through a freeze-drying crosslinking technique. This NC aerogel filter successfully separated oil and water in emulsions, achieving 100% efficiency even after 10 cycles of use and producing a 98.6% oil-free water surface emulsion [55]. Free-standing nanocellulose films have shown promise in the field of oil and water separation due to their unique properties. These films are derived from renewable sources, making them environmentally friendly. The high surface area and porosity of nanocellulose films allow for efficient adsorption of oil molecules while repelling water molecules, leading to the effective separation of the two liquids. The hydrophobic/hydrophilic nature of nanocellulose films can be tailored through surface modification or functionalization to enhance their performance in oil and water separation applications. Additionally, the mechanical strength and flexibility of these films make them ideal for practical use in separating oil and water mixtures. The lightweight and cost-effective nature of nanocellulose films further adds to their appeal for large-scale applications in industries such as wastewater treatment, oil spill cleanup, and oil-water separation processes in manufacturing plants. Overall, free-standing nanocellulose films offer a sustainable and efficient solution for separating oil and water, showcasing the potential of nanocellulose-based materials in environmental remediation and industrial processes.

4.3. Photocatalyst composite

Free-standing nanocellulose films are a promising material for use in photocatalyst composites. These films offer a high surface area and excellent mechanical properties, making them suitable for various applications in photocatalysis. Nanocellulose, derived from renewable sources like wood pulp, is biodegradable and environmentally friendly. When incorporated into photocatalyst composites, nanocellulose films can enhance the overall performance of the material. Their large surface area provides more active sites for catalytic reactions, while their mechanical strength adds stability to the composite structure. Additionally, nanocellulose can act as a scaffold for supporting photocatalytic particles, such as titanium dioxide or other semiconductors, improving their dispersion and efficiency. The free-standing nature of these films allows for easy handling and integration into different systems without the need for additional support materials. This makes them versatile for various applications, such as water purification, air treatment, and energy conversion. Overall, the combination of nanocellulose films with photocatalysts in composites holds great potential for creating sustainable and effective materials for environmental remediation and energy production. This composite material was created by combining nanocellulose, polyamidoamine-epichlorohydrin (PAE), and titanium dioxide (TiO_2) nanoparticles through vacuum filtration. It was specifically engineered as a photocatalyst for the purification of wastewater. As illustrated in **Figure 6**, the titanium dioxide component within the composite effectively breaks down 95% of methyl orange dye present in water within a 150-minute timeframe when exposed to ultraviolet (UV) light. The network of nanocellulose fibers in conjunction with PAE serves to securely retain the TiO_2 nanoparticles, thereby preventing their dispersion into the wastewater. This composite material exhibits potential for application as a cost-effective and reusable photocatalytic membrane in the treatment of wastewater [56].

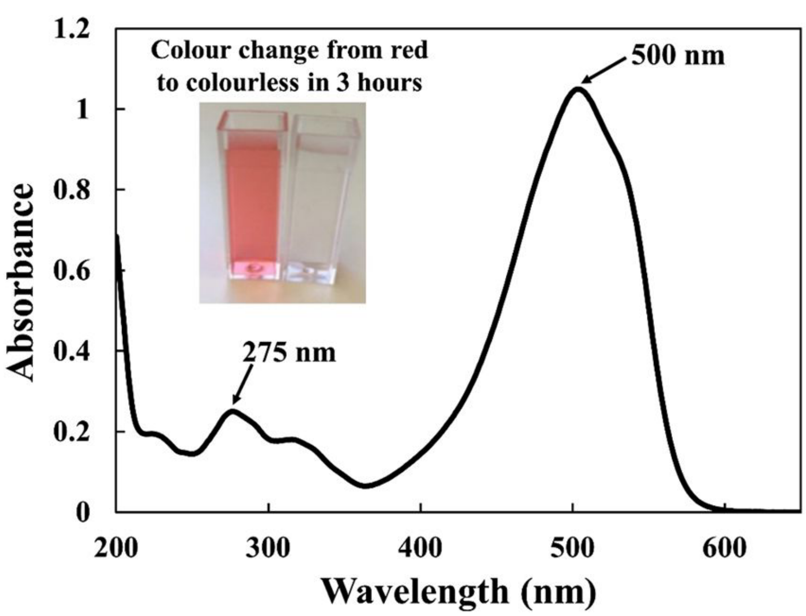

Figure 6. The degradation of methyl orange solution by TiO_2-MFC composite as photocatalyst. The spectrum indicates two absorption maxima. The color of dye solution was reduced using photocatalysis composite [56].

4.4. Membrane

Free-standing nanocellulose films have shown promising potential as membranes for water and wastewater treatment due to their eco-friendly nature, high mechanical strength, and tunable porosity. These films can effectively filter out contaminants such as heavy metals, organic pollutants, and bacteria, making them ideal for various filtration applications. Nanocellulose membranes exhibit high water flux and selectivity, making them suitable for desalination processes, removal of micropollutants, and separation of oil and water emulsions. Their sustainable and biodegradable nature further enhances their appeal for environmental applications. The unique properties of nanocellulose, such as its high surface area, hydrophilicity, and chemical functionality, can be tailored to enhance membrane performance and durability. Additionally, these membranes can be easily integrated into existing water treatment systems, offering a cost-effective and sustainable solution for clean water production. Overall, free-standing nanocellulose films hold great promise as membranes for water and wastewater treatment, contributing to the development of efficient and environmentally friendly filtration technologies.

Cellulose nanofiber membranes offer a sustainable alternative to synthetic plastic membranes due to their recyclability and biodegradability. In this study, a novel composite material was fabricated through vacuum filtration, incorporating nanocellulose, silica nanoparticles, and PAE. The silica nanoparticles, with a diameter of 22 nm, served as spacers to regulate the porosity of the composite. PAE was included in the composite to interact with the negatively charged silica nanoparticles, enhancing the wet strength of the material. The resulting composite membrane exhibited a water flux of 80 L/m^2/hour and a molecular weight cut-off of 200 kDa. By adjusting the quantity of silica nanoparticles, the pore size of the membrane can be customized. This composite membrane functions effectively as a filtration membrane, particularly suitable for ultrafiltration applications [57].

4.5. Gels

Nanocellulose gels are advanced materials that are produced from nanocellulose, which is a renewable and biodegradable nanomaterial derived from cellulose fibers. These gels have gained significant interest in various industries due to their unique properties and potential applications. Nanocellulose gels exhibit excellent mechanical strength, high water-holding capacity, and biocompatibility, making them suitable for a wide range of applications. In the food industry, nanocellulose gels can be used as thickeners, stabilizers, or emulsifiers due to their ability to enhance the texture and stability of food products. In the pharmaceutical and cosmetic industries, they can be utilized in drug delivery systems, wound dressings, and skin care products due to their biocompatibility and ability to retain moisture. Furthermore, nanocellulose gels have also shown promise in environmental applications such as water purification and remediation due to their adsorption capabilities and environmentally friendly nature. Overall, nanocellulose gels represent a promising class of materials with a wide range of applications across various industries.

Nanocellulose has the capability to be transformed into hydrogels and gels suitable for various biological and medical purposes through the TEMPO-oxidation technique. These nanocellulose gels have potential applications in protein separation using electrophoresis and in bioseparation diagnostics [58]. Nanocellulose hydrogels and gels can serve as appropriate foundations for 3D cell cultivation to establish a microenvironment conducive to cell proliferation, as well as for the development of tissue engineering scaffolds and drug delivery carriers [59]. By subjecting carboxylated nanocellulose to TEMPO-oxidation, it can be transformed into foam structures, functioning as a highly absorbent material known as super adsorbent material [59,60].

4.6. Anti-microbial nanocomposites

Anti-microbial nanocomposites derived from nanocellulose offer a promising solution for combating microbial growth in various applications. By incorporating antimicrobial agents into nanocellulose matrices, these nanocomposites exhibit enhanced antimicrobial properties due to their high surface area-to-volume ratio and unique structural properties. Nanocellulose-based antimicrobial materials have shown great potential in a wide range of fields, including food packaging, medical devices, wound dressings, water treatment, and cosmetics. The antimicrobial activity of these nanocomposites can be tailored by selecting specific antimicrobial agents and adjusting their concentration in the nanocellulose matrix. Additionally, the biocompatibility and biodegradability of nanocellulose make it an attractive choice for developing sustainable antimicrobial materials. These nanocomposites have the potential to reduce the need for traditional antimicrobial agents that may have negative environmental impacts. Overall, anti-microbial nanocomposites derived from nanocellulose represent a promising avenue for developing innovative and sustainable solutions to combat microbial contamination in various industries while also addressing the growing concern of antimicrobial resistance.

Nanocellulose composites with antimicrobial properties were created using an antimicrobial agent known as phenyl bismuth bis(diphenyl phosphinate) through vacuum filtration and spraying techniques. These composites have the potential to serve as antimicrobial packaging materials, biomedical bandages, and antimicrobial coatings. Recent studies have demonstrated that bismuth-based antimicrobial agents exhibit a wide spectrum of activity against various pathogens, including those that are resistant to antimicrobials. The antimicrobial efficacy of the composite containing 5 wt% bismuth was evidenced by a 15 mm zone of inhibition against both Gram-negative microorganisms like *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*), Gram-positive bacteria such as *Staphylococcus aureus* (*S. aureus*), and antimicrobial-resistant pathogens such as vancomycin-resistant *Enterococcus* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA). Furthermore, the water vapor permeability of this composite was determined to be 4.4 x 10-11 g/Pa.s.m, a value comparable to that of synthetic plastics [61,62].

4.7. High-performance nanocomposites

Nanocellulose-based composites were developed for the purpose of creating high-performance barrier materials using an *in situ* precipitation technique. This method involved the precipitation of calcium carbonate nanoparticles within a nanocomposite film through a chemical reaction between sodium carbonate and calcium chloride. The resulting porous composite exhibited low permeability due to the reduction in pore volume caused by the precipitated nanoparticles. A comparison between a pure nanocomposite film and the in-situ precipitated composite, prepared from 0.2 M solutions with $CaCO_3$ nanoparticles embedded within, is illustrated in **Figure 7**. The water vapor transmission rate and oxygen transmission rate of the composite containing 1 wt% $CaCO_3$ precipitated in the nanocomposite film were measured at 4.7 g/m^2.day and 2.7 cc/m^2.day, respectively, at 23°C and 50% relative humidity. Additionally, the tensile strength and E-Modulus of this composite were determined to be 91.96 ± 14.99 MPa and 4.6 GPa, respectively, indicating favorable strength and stiffness properties [54].

Highly efficient membranes were successfully produced using a nanocellulose-silicon dioxide, SiO_2 composite through the conventional vacuum filtration technique. The composite exhibited a pore size of less than 100 nm, and its porosity could be adjusted by varying the SiO_2 content in the NC suspension [63]. Additionally, an NC-montmorillonite (MMT) composite was created using vacuum filtration for packaging purposes. The water vapor permeability of the blended NC-MMT composite was measured at 6.33 ± 1.5 g.µm/m^2.day.kPa with 23.1 wt% MMT, demonstrating comparable performance to synthetic plastics [4]. **Table 3** shows the summary of the application of nanocellulose.

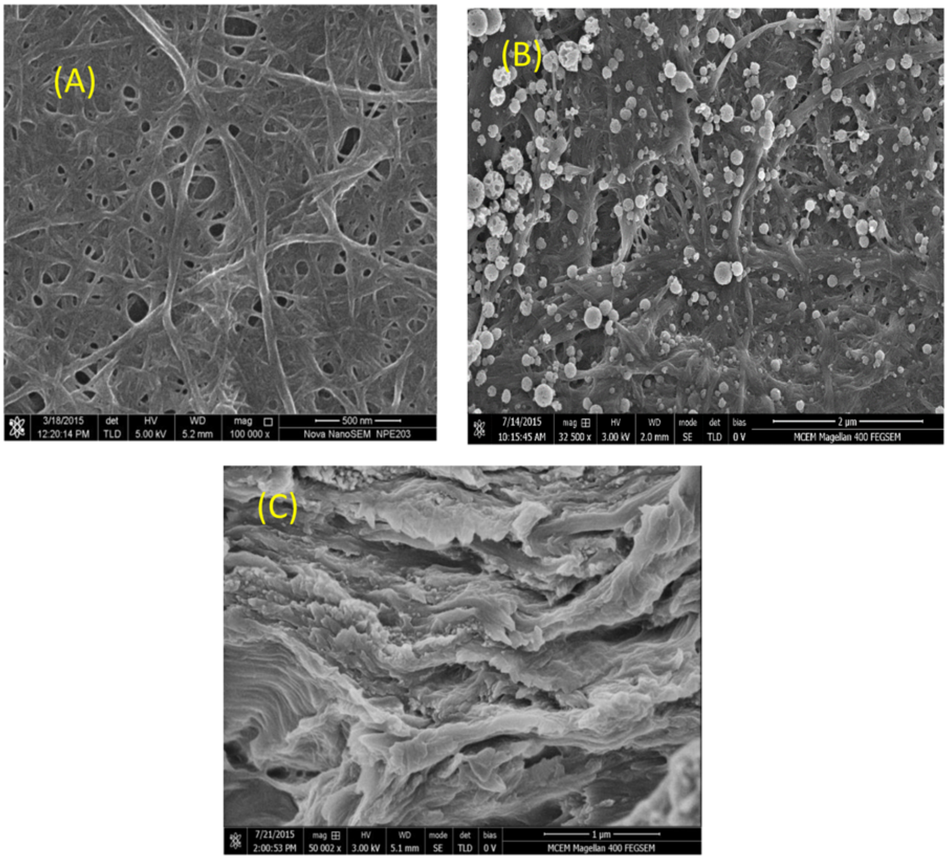

Figure 7. SEM micrographs of CNF film and its composite via *in situ* precipitation of nanoparticles. (A) Pure CNC film. (B, C) Surface and cross-sectional view of the composite prepared via *in situ* precipitation [54].

Table 3. Application of nanocellulose [10]

High consumption of NC	Low consumption of NC	Novel applications and those under development
Paper production process Strength additives Barrier layers Glossy surface Reduction of paper grammage Produce smart packaging Binding filler	Insulators Protection against sound and heat	Medical, industrial, and environmental areas Biodegradable sensor membranes for air and water filtration
Coating and films Co-binder and thickener for paper coating Nanofiltration Membranes Barrier films	Aerospace industries Advanced composite materials Structures interiors	Construction field Reinforcement fibers
Cement processing Concrete additive	Petroleum Aerogels Oil and gas processing	Electronics Smooth substrates for flexible electronics Conducting substrates Recyclable organics LED photovoltaics
Automotive industry Composite materials	Rheological modifier Functional pigments and paint products	Cosmetics

Table 3 (Continued)

High consumption of NC	Low consumption of NC	Novel applications and those under development
Packaging Composites Films Fillers	Construction composites	Packaging Intelligent barrier materials
Printing paper Good quality of printing		Films for photonics
Cosmetics Hygiene and absorbent products		3D printing Tissue engineering scaffold
Textiles		

5. Nanocellulose-MMT composites and their applications

Nanocellulose is a promising material derived from plant fibers, possessing unique properties such as high strength, low density, and biodegradability. When combined with montmorillonite, a type of clay mineral, nanocellulose can form nanocomposites with enhanced mechanical and barrier properties. Nanocellulose-montmorillonite nanocomposites have shown great potential in various applications due to their improved strength, stiffness, and thermal stability. These nanocomposites can be used in industries such as packaging, aerospace, automotive, and biomedicine. The combination of nanocellulose and montmorillonite creates a synergistic effect, leading to superior performance compared to individual components. The nanocellulose provides reinforcement and stiffness, while montmorillonite contributes to improved barrier properties and thermal stability. Overall, nanocellulose-montmorillonite nanocomposites offer a sustainable and eco-friendly alternative to traditional materials, with the potential to revolutionize various industries with their unique properties and versatility. Despite its advantageous properties such as flexibility, strength, biodegradability, and sustainability, NC material exhibits limitations in oxygen and water vapor permeability, particularly at elevated humidity levels. This is attributed to the enlargement of pore size in NC film caused by the swelling of cellulose nanofibrils. The formation of flocs during the preparation of nanocellulose films presents a challenge. To address this issue in NC film production, researchers have explored strategies such as combining NC with polyelectrolytes or nano inorganics to create composite materials [4,18].

One significant application of NC is the development of composites containing nano-inorganics. The nano inorganic platelets engage with the network of cellulose nanofibrils and occupy their pores, resulting in composites with significantly improved properties. These composites find specific utility in various applications such as barriers, fire retardants, membranes, and heat-insulating materials. The addition of nanoclay to the NC fiber network enhances both its mechanical and barrier characteristics. The interaction between nano-inorganics and cellulose nanofibrils serves to enhance the functionality of the composite [4,64,65]. The performance of composites can be improved through various methods such as filling the pores within the cellulose fibrous network to create effective barrier composites, forming aggregates within the cellulose network to produce fire retardant materials, or adjusting the pores of the cellulose fibrous network for membrane applications [57,63,66]. The potential applications of nanocellulose-based composites are outlined in **Table 4**.

5.1. Barrier coating

Nanocellulose, specifically MMT, has gained interest for barrier coating applications on paper and paperboard due to its unique properties. Montmorillonite, a type of clay mineral, can improve the barrier properties of nanocellulose coatings by enhancing its mechanical strength and water resistance. These coatings can provide better protection against moisture, oxygen, and grease, extending the shelf life of packaged products. Nanocellulose-MMT coatings offer a sustainable and environmentally friendly alternative to traditional petroleum-based barrier coatings. The combination of these two materials can create a biodegradable and recyclable barrier coating that reduces the environmental impact of packaging materials. Additionally, nanocellulose coatings can contribute to the lightweight of packaging, helping to reduce transportation costs and carbon emissions. Overall, the use of nanocellulose-MMT coatings for barrier applications on paper and paperboard shows great promise in improving the performance and sustainability of packaging materials. Researchers and industries are exploring this innovative technology to develop more efficient and eco-friendly packaging solutions for various applications.

Nanocellulose-MMT suspension has the potential to be utilized in coating applications to create a composite barrier layer on cellulose substrates. This composite barrier coating effectively reduces the permeability of oxygen and water vapor through the sheet. For instance, when a base sheet with a thickness of 22 ± 0.5 μm and a coat weight of 29 ± 0.5 g/m^2 is coated with 5 wt% MMT and 1.4 wt% NC, the resulting barrier exhibits a water vapor transmission rate (WVTR) of 8 ± 0.3 g/m^2 day and an oxygen transmission rate (OTR) of 36400 ± 1100 cm^3/m^2 day. In contrast, applying only 1.4 wt% NC coating on a base sheet with a thickness of 11 ± 0.3 μm and a coat weight of 9.9 ± 0.2 g/m^2 led to a WVTR of 24 ± 0.7 g/m^2 day, with the OTR exceeding the measurable range. Therefore, the introduction of 5 wt% MMT in the NC suspension results in a notable reduction in WVTR and brings the OTR within a measurable range [67].

5.2. Membrane

Nanocellulose-montmorillonite composites have shown great potential as membranes for water and wastewater treatment due to their unique structural and functional properties. Nanocellulose, derived from plant-based sources, offers high mechanical strength, flexibility, and biodegradability. Montmorillonite, a type of clay mineral, provides excellent adsorption capacity and ion exchange properties. When combined, nanocellulose and montmorillonite create a synergistic effect, enhancing the overall performance of the membrane. The nanocellulose matrix can provide a stable support structure, while the montmorillonite particles can improve the membrane's permeability and selectivity. These composite membranes have been found to effectively remove contaminants such as heavy metals, dyes, and organic pollutants from water and wastewater streams. Their high surface area and porosity make them efficient in filtration processes, while their sustainable and environmentally friendly nature makes them attractive for various applications. Overall, the use of nanocellulose-montmorillonite composites as membranes for water and wastewater treatment holds great promise for addressing water scarcity and pollution challenges while ensuring sustainability and efficiency in water treatment processes. Nanocellulose-montmorillonite has the potential to be employed in the fabrication of membranes. One application involves incorporating montmorillonite into membranes to facilitate the adsorption of cationic dyes present in wastewater, serving as a cation exchanger for water treatment purposes. Furthermore, these composite materials have shown promise in the removal of heavy metals from wastewater [68,69].

Table 4. Application of NC-based nanocomposites [70]

Field of applications	Functions of nanocomposites
Composites and plastics	Shelf life extension Heat resistance Dimensional stability
Paper and packaging	Intelligent packaging See-through packaging Ultra-violet screening packaging Anti-microbial packaging
Medical application	Drug delivery and controlled release Scaffold in tissue engineering Implants
Barrier properties	Shelf life extension Down-gauging of films Oxygen scavenger
Electronics	Time-temperature integrator Freshness indicator Gas and leakage detector Sensors for food monitoring Signal processor for biochemical pathways
Aerogel	Self-healing material

5.3. Flame and fire-retardant

Nanocellulose-montmorillonite composite materials have shown great potential as flame and fire-retardant materials due to their unique properties. The combination of nanocellulose, derived from renewable sources like plants, and montmorillonite, a natural clay mineral, results in a synergistic effect that enhances the flame retardancy of the composite. Nanocellulose provides excellent mechanical properties and forms a protective char layer when exposed to heat, which can act as a barrier against flames. Montmorillonite, on the other hand, has a high aspect ratio and thermal stability, further contributing to the fire-retardant properties of the composite. These materials have low thermal conductivity and are capable of reducing heat transfer, thus delaying the spread of flames. Additionally, nanocellulose-montmorillonite composites are lightweight, environmentally friendly, and cost-effective compared to traditional flame retardants. Overall, the combination of nanocellulose and montmorillonite offers a promising solution for developing flame and fire-retardant materials with enhanced performance and sustainability. Further research and development in this area can lead to innovative applications in various industries, such as construction, textiles, and electronics.

The creation of environmentally friendly and sustainable flame and fire retardants as substitutes for synthetic materials presents a complex challenge. Cellulose-based flame- and fire-retardant substances offer a sustainable and eco-friendly option compared to halogen-based retardants, which pose a toxic threat by introducing halogen compounds into the food chain. Recent research has demonstrated that nanocellulose can effectively serve as a foundational material for flame-retardant substances in the form of nanocomposites. By incorporating clay platelets into the nanocellulose fiber network, a composite material is formed that enhances fire resistance. The fibrous structure of the network contributes to the durability and robustness of the composite material [66,70]. The incorporation of nanoclay represents an alternative method to customize the characteristics of composites in order to enhance their flame-retardant properties [66]. For instance, the oxygen permeability (OP) of a 5 wt% MMT composite film fabricated through casting is measured at 0.006 mL μm m^{-2} day^{-1} kPa^{-}

[1] under 0% relative humidity (RH), with mechanical properties showing an E-Modulus of 18 GPa and a tensile strength of 509 MPa. By increasing the MMT content to 50 wt% in the composite, the OP is reduced to 0.0008 mL μm m^{-2} day^{-1} kPa^{-1}. However, the mechanical strength of the composite film diminishes with a 50 wt% MMT loading. These composites have potential applications as gas barrier materials [70]. The study examined a composite material consisting of 50 wt% montmorillonite (MMT) and 50 wt% nanocellulose arranged in a continuous fibrous network through vacuum filtration. This composite exhibited a tensile strength of 124 MPa and a Young's modulus of 8.7 GPa. The oxygen transmission rate (OTR) of the pure NC film was measured at 0.048 cm^3 mm m^{-2} day^{-1} atm^{-1} at 50% relative humidity (RH) and 17.8 cm^3 mm m^{-2} day^{-1} atm^{-1} at 95% RH. In comparison, the OTR of the 50 wt% MMT–50 wt% NC composite was found to be 0.045 and 3.5 cm^3 mm m^{-2} day^{-1} atm^{-1} at 50% and 95% RH, respectively. The oxygen permeability (OP) of the pure NC film increased by 370% from 50% RH to 95% RH, while the composite showed only a 13% increase in OP over the same RH range. This suggests that the incorporation of MMT into the fibrous structure resulted in a composite material with potential gas barrier and flame-retardant properties. Both 30 wt% and 50 wt% MMT in the NC composite demonstrated effective fire retardancy, as confirmed by flammability tests and calorimetry, indicating self-extinguishing characteristics [66]. The oriented MMT within the NC fibrous network was observed to impede the diffusion of oxygen and the ignition process [70].

6. Conclusion

Recently, nanocellulose films and their composites have shown promise for a variety of applications across different industries. These films have been primarily utilized in functions such as barriers, air filtration, antimicrobial coatings, substrates for electronic devices, and light-emitting diodes, among others, with the aim of replacing conventional synthetic plastics. However, the current methods for producing nanocellulose films are time-consuming and have limited production rates, impacting the properties of the resulting films and composites. This review highlights the necessity for a more efficient and rapid production process for NC films and their composites, emphasizing the importance of flexibility in tailoring the properties of these materials to suit specific functionalities. Films and composites from nanocellulose offer a wide range of properties and applications, making them attractive materials for various industries seeking sustainable and functional packaging solutions, biomedical devices, and advanced materials. Given this correspondence, nanocellulose can be a potential eco-friendly nanomaterial to replace synthetic plastics in conventional practice.

Disclosure statement

The author declares no conflict of interest.

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Exploring the Role of CDKN2A in Human Cancers Using an Integrative Pan-Cancer Approach

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Abstract: This study aims to investigate the expression variation, biological significance, and prognostic value of cyclin-dependent kinase inhibitor 2A (CDKN2A) as a common biomarker across 33 malignancies. Various bioinformatics tools, including UALCAN, GEPIA2, OncoDB, cBioPortal, TIMER2, STRING, DAVID, and the GSCA database, were employed for this pan-cancer analysis. The results revealed significant up-regulation of CDKN2A in 24 major human cancer subtypes ($P < 0.05$). This up-regulation was strongly associated with poor overall survival and tumor dissemination, particularly in uterine corpus endometrial carcinoma (UCEC), colon adenocarcinoma (COAD), and liver hepatocellular carcinoma (LIHC), highlighting its potential as a prognostic biomarker. Moreover, CDKN2A overexpression was linked to diverse clinicopathological characteristics of patients. Genetic alterations recorded via cBioPortal indicated minimal mutation rates in COAD, LIHC, and UCEC. Additionally, promoter methylation, drug sensitivity, and enrichment analyses were performed to explore associations with CDKN2A expression. Overall, the findings emphasize the potential of CDKN2A as a shared diagnostic and prognostic biomarker, as well as a therapeutic target in COAD, LIHC, and UCEC, particularly in patients with varied clinicopathological traits.
Keywords: CDKN2A; Cancer; Prognosis biomarker; Therapeutic target

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1. Introduction

Cancer, a complex and diverse group of diseases, remains a critical public health concern and the leading cause of mortality worldwide [1-3]. It is characterized by the uncontrolled proliferation and dissemination of abnormal cells, significant heterogeneity, intricate genomic alterations, and a wide array of molecular abnormalities that affect nearly every tissue and organ [4-6]. Global statistics indicate that the number of new cancer cases and fatalities rose to 20 million and 9.7 million, respectively, in 2022, compared to 18.1 million and 9.6 million in 2018 [7,8]. The multifactorial etiology of cancer involves the interaction of genetic variants, environmental exposures, and lifestyle factors [9-11]. Despite significant advancements in cancer diagnostics and treatments, including immunotherapy, targeted therapies, chemoradiotherapy, and surgery, the prognosis for cancer patients remains poor, primarily due to tumor heterogeneity, distant metastases, acquired drug resistance, and recurrence [12-14]. As a result, the identification of novel diagnostic and prognostic biomarkers for various cancers is of paramount importance.

Cyclin-dependent kinase inhibitor 2A (CDKN2A), located on band p21.3 of human chromosome 9, is ubiquitously expressed in many tissues, including the adrenal glands, bladder, testis, stomach, spleen, and fat tissue [15,16]. CDKN2A functions as a tumor suppressor gene encoding two proteins, p14ARF and p16INK4a. These proteins play key roles in cell cycle regulation; p16INK4a inhibits CDK4, inducing G1 cell cycle arrest and preventing retinoblastoma phosphorylation, while p14ARF activates p53 [17-19]. Deletions, insertions, point mutations, and epigenetic changes are among the most common alterations of CDKN2A, with its deletion identified in 1.7% to 6.7% of cases, often associated with various cancers. Homozygous deletions of CDKN2A are linked to poor prognosis in IDH-mutant gliomas, supratentorial ependymomas, meningiomas, and malignant peripheral nerve sheath tumors (MPNST), and have been recognized as diagnostic criteria by the World Health Organization [20-23].

Single nucleotide polymorphisms (SNPs) in the p14ARF protein can also lead to different cancer types [24]. CDKN2A plays a significant role in inherited cancers, particularly in familial atypical multiple-mole melanoma (FAMMM), which increases the risk of melanoma and pancreatic cancer [25]. Alterations in CDKN2A, such as point mutations, translocations, homozygous and heterozygous losses, and abnormal promoter methylation, have been associated with melanoma, non-small cell lung cancer (NSCLC), head and neck cancers, prostate, esophageal, ovarian, kidney, colon, breast, and bladder cancers [26-28]. Moreover, aberrant CDKN2A expression correlates closely with immune infiltration and immune-regulatory gene levels. Several activated immune cells show a strong positive correlation with high CDKN2A expression, suggesting an intriguing role for CDKN2A in tumor immunity.

CDKN2A exhibits significant expression across various cancers, including breast cancer (BRCA), head and neck squamous cell carcinoma (HNSC), colon adenocarcinoma (COAD), kidney renal cell carcinoma (KIRC), stomach adenocarcinoma (STAD), lung adenocarcinoma (LUAD), liver hepatocellular carcinoma (LIHC), and uterine corpus endometrial carcinoma (UCEC) [29-31]. These findings indicate that CDKN2A may serve as a key target for cancer diagnosis and therapeutic intervention, given its critical involvement in cancer progression. However, the precise role of CDKN2A in pan-cancer contexts remains unclear.

This study aims to analyze CDKN2A expression across multiple human cancer subtypes and its association with various parameters, including promoter methylation levels, overall survival (OS), relapse-free survival (RFS), genetic mutations, copy number variations (CNVs), immune cell infiltration, gene enrichment, and gene-drug interaction networks, using several online databases and bioinformatics tools.

2. Materials and methods

2.1. Pan-cancer expression analysis of CDKN2A

UALCAN is an online database developed for the comprehensive examination of cancer-associated data across several cancer subtypes [32]. In this study, UALCAN's default settings were utilized to perform a pan-cancer analysis of CDKN2A expression in both tumor and normal samples, based on different parameters across 33 cancer types. A P-value of <0.05 was considered statistically significant.

2.2. Survival analysis

GEPIA2 is a robust bioinformatics tool designed to investigate variations in gene expression within different tissues and tumor types [33]. In the present study, the impact of CDKN2A expression on OS in various cancers was evaluated using the Survival Plot module of GEPIA2, with a P-value of < 0.05 set as statistically significant.

2.3. Promoter methylation analysis

OncoDB is an essential database functioning as a comprehensive platform for oncogenic mutations, with a specific emphasis on DNA methylation data [34]. In this study, OncoDB was employed to investigate the promoter methylation levels of CDKN2A across various cancers, enabling the identification of its potential as a biomarker.

2.4. Mutational analysis using cBioPortal

cBioPortal is a web-based application designed for the assessment of genetic alterations across numerous cancers [35]. In this study, cBioPortal's advanced features were used to conduct a thorough mutational characterization of the CDKN2A gene across diverse cancer subtypes.

2.5. Immunogenetics analysis

The relationship between immune cell infiltration and gene expression across distinct cancer types can be evaluated using the Tumor Immune Estimation Resource (TIMER2) database [36]. In this study, the associations between CDKN2A expression and the infiltration levels of various immune cell populations were analyzed using the TIMER2 algorithm.

2.6. Protein-protein interaction network and enrichment analysis

Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) is a significant bioinformatics resource that facilitates the visualization of protein-protein interaction networks [37]. In this study, the protein-protein interaction (PPI) network corresponding to CDKN2A and its interacting proteins was constructed using the STRING database. Database for Annotation, Visualization, and Integrated Discovery (DAVID) is another bioinformatics tool used to elucidate the functional significance of gene lists [38]. Gene enrichment analysis of CDKN2A was performed using the DAVID tool.

2.7. Drug sensitivity analysis

The GSCA database is a valuable resource for assessing pharmacological sensitivity [39]. In this study, the correlation between drug sensitivity and the mRNA expression of CDKN2A was explored using the GSCA database.

3. Results

3.1. Pan-cancer expression analysis of CDKN2A

The pan-cancer analysis of CDKN2A expression across 33 cancers was conducted using the TCGA and GTEx databases through UALCAN. The results demonstrated that CDKN2A was significantly ($P < 0.05$) overexpressed in 24 cancer subtypes, including squamous-cell carcinoma (SCC) of the lung, bladder urothelial carcinoma (BLCA), papillary renal cell carcinoma (PRCC), esophageal carcinoma (ESCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), stomach adenocarcinoma (STAD), BRCA, LIHC, COAD, HNSC, and UCEC (**Figure 1**).

3.2. Prognostic analysis of CDKN2A

GEPIA2 was utilized to further assess the role of CDKN2A overexpression on OS across various cancer types. The results indicated that upregulated CDKN2A expression was significantly ($P < 0.05$) correlated with worse OS and RFS in five cancer subtypes, including adenoid cystic carcinoma (ACC), COAD, LIHC, UCEC, and uveal melanoma (UVM) (**Figure 2**). This association of upregulated CDKN2A expression with poor OS in ACC, COAD, LIHC, UCEC, and UVM highlights the potential of CDKN2A as a prognostic biomarker.

3.3. CDKN2A expression in COAD, LIHC, and UCEC patients categorized by various features

The UALCAN database was employed to investigate CDKN2A expression in COAD, LIHC, and UCEC samples, stratified by attributes such as patient age, ethnicity, and clinical stages. Significant (P-value < 0.05) upregulation of CDKN2A expression was observed, and this expression demonstrated correlations with clinicopathological characteristics, including patient age, ethnicity, and cancer staging (**Figure 3**).

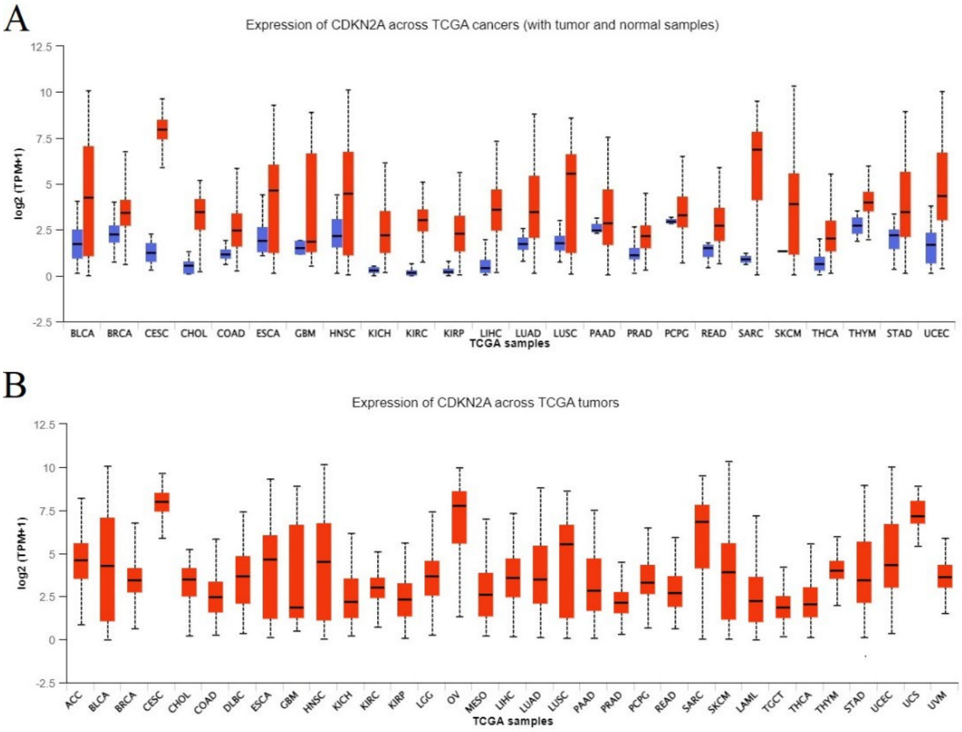

Figure 1. Differential transcription expression analysis of the CDKN2A gene via pan-cancer analysis using UALCAN from the TCGA database. (A) Analysis of CDKN2A between tumor and normal samples via UALCAN from the TCGA database. (B) Pan-cancer expression analysis results of CDKN2A in cancerous samples. *P < 0.05 was considered significant

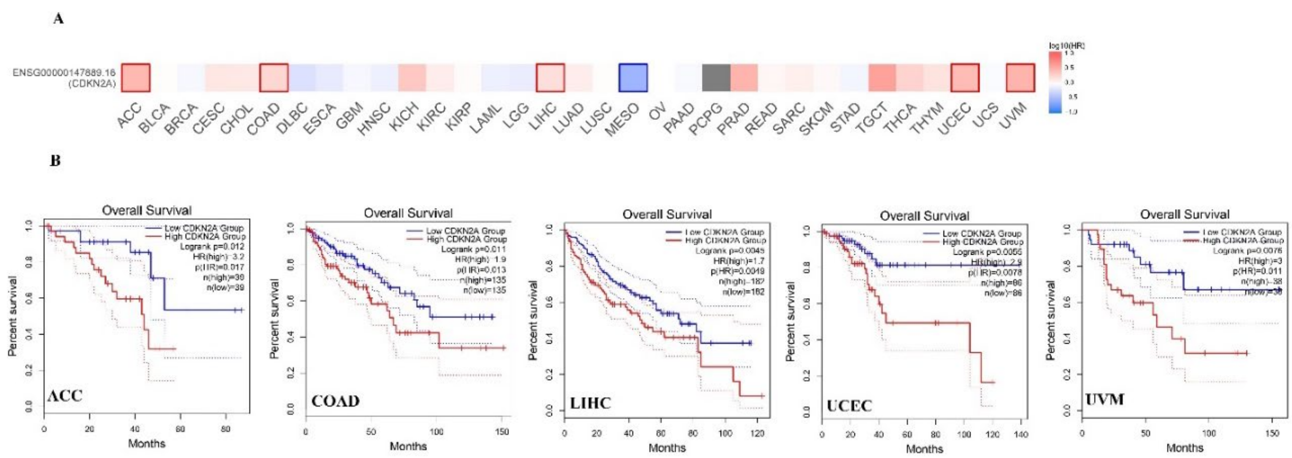

Figure 2. Pan-cancer prognosis analysis of CDKN2A expression. (A) The survival map based on CDKN2A expression across 33 cancer types. (B) The impact of CDKN2A expression on overall survival. *P-value < 0.05

3.4. Analysis of CDKN2A promoter methylation levels

Dysregulated DNA methylation, including localized hypermethylation and genomic hypomethylation, is associated with various diseases, particularly cancer [40,41]. In this study, the UALCAN database was employed to examine CDKN2A promoter methylation levels in COAD, LIHC, and UCEC samples compared to normal tissues. The results indicated that CDKN2A was hypomethylated in COAD, LIHC, and UCEC samples, revealing a positive correlation between CDKN2A methylation and its expression levels (**Figure 4**).

3.5. Genetic alterations of CDKN2A

Genetic alterations are linked to cancer progression. In this study, cBioPortal was used to assess genetic modifications related to CDKN2A in COAD, LIHC, and UCEC samples. The results showed that CDKN2A mutations occurred in 1.8% of COAD, 8% of LIHC, and 0.8% of UCEC samples, with observed mutations including truncating, missense, amplification, and deep deletion (**Figure 5**). These findings provide insights into potential mutational pathways underlying cancer progression.

3.6. Analysis of immune cell infiltration

Immune cell infiltration significantly influences cancer initiation and progression [42]. TIMER2.0 was used to evaluate the relationship between CDKN2A expression and immune cell infiltration, including $CD8^+$ T cells, $CD4^+$ T cells, and B cells. In UCEC, an inverse relationship between CDKN2A expression and $CD8^+$ and $CD4^+$ T cells was observed, while weak correlations were found in LIHC and COAD (**Figure 6A–B**). Additionally, no correlation was detected between CDKN2A expression and B cell infiltration in UCEC, COAD, and LIHC (**Figure 6C**).

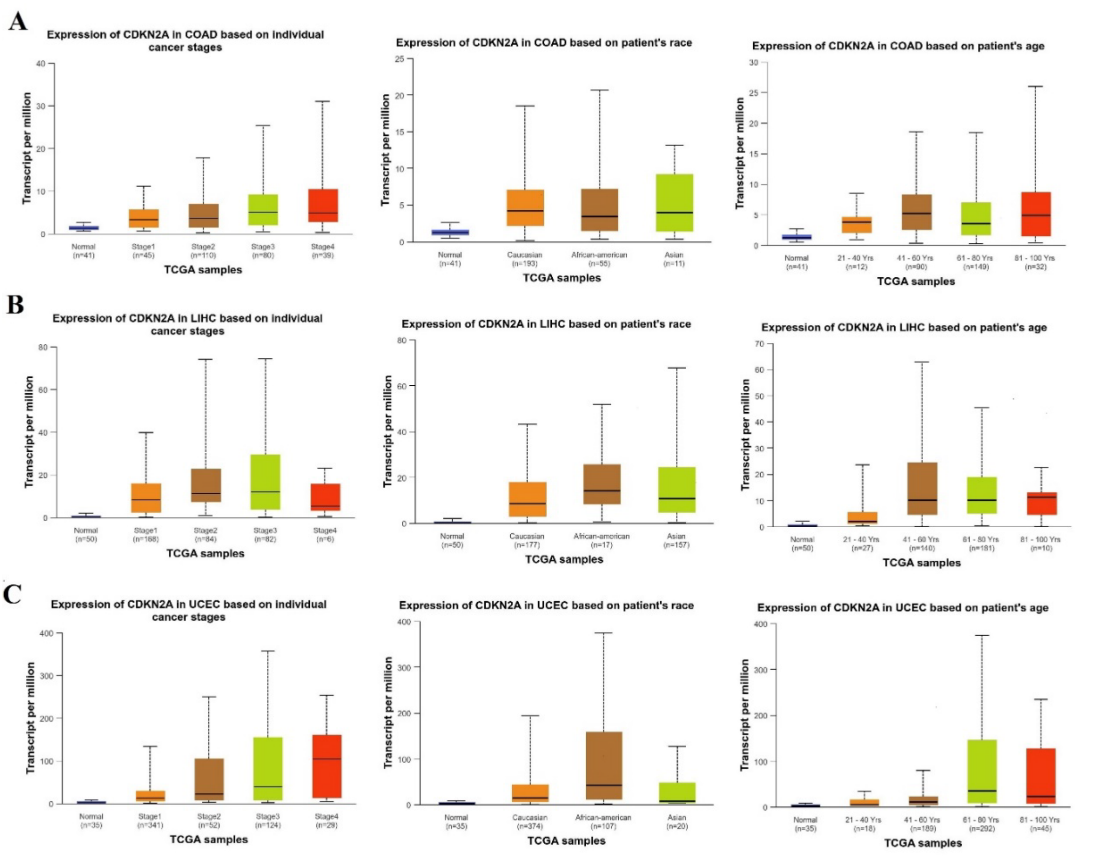

Figure 3. CDKN2A expression in COAD, LIHC, and UCEC samples based on various attributes, including patient age, race, and cancer stage. (A) CDKN2A expression in COAD samples. (B) CDKN2A expression in LIHC samples. (C) CDKN2A expression in UCEC samples

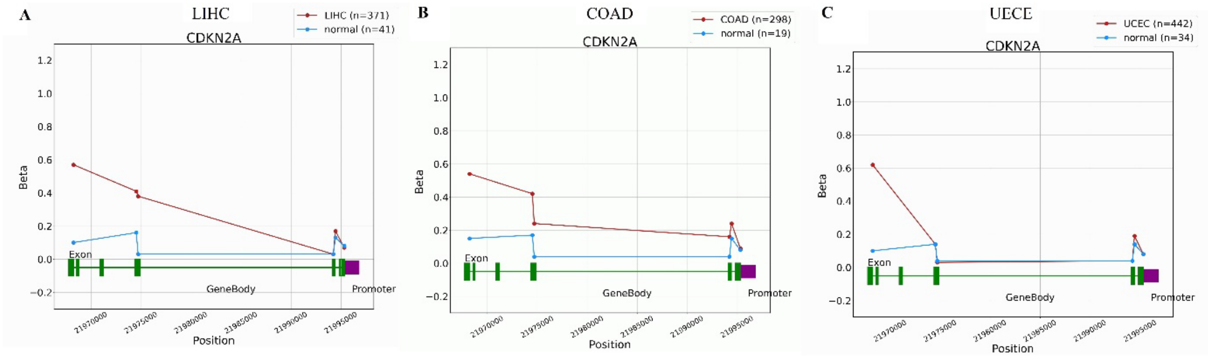

Figure 4. Correlation between CDKN2A mRNA expression and promoter methylation status in COAD, LIHC, and UCEC using the OncoDB database. Significance level = P-value < 0.05

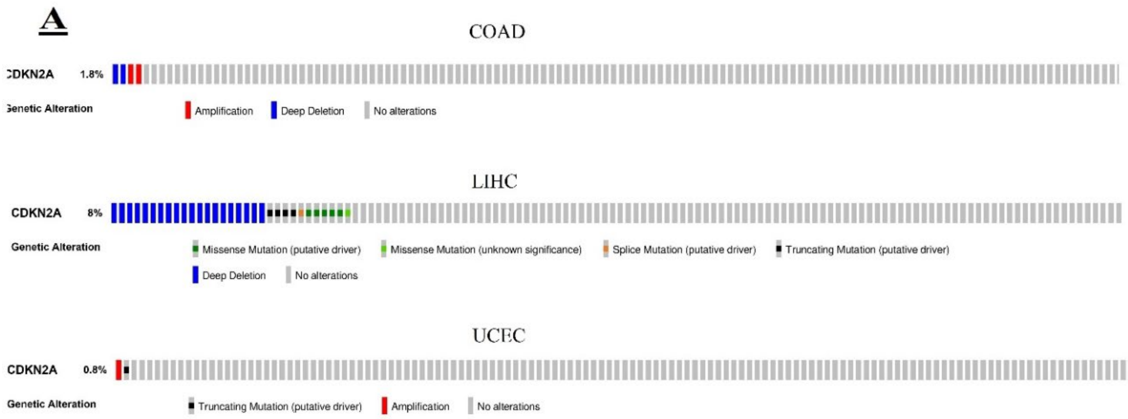

Figure 5. Insights into the mutational pathways underlying the progression of COAD, LIHC, and UCEC cancers

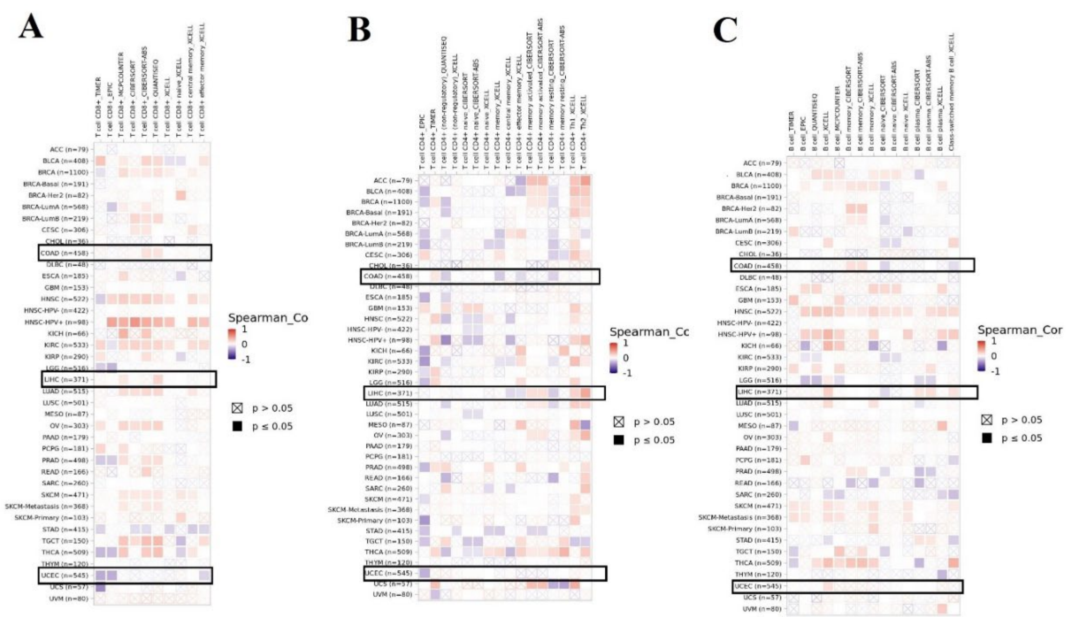

Figure 6. Correlation between CDKN2A expression and immune cell infiltration in COAD, LIHC, and UCEC samples. (A) Correlation with $CD8^+$ T cell infiltration. **(B)** Correlation with $CD4^+$ T cell infiltration. **(C)** Correlation with B cell infiltration. *P-value < 0.05.

3.7. Protein-protein interaction (PPI) network and gene enrichment analysis

Further analysis was conducted to explore the molecular mechanisms associated with CDKN2A. The PPI network revealed ten CDKN2A-associated proteins: TP53, CCND1, CCND2, MDM2, CDK6, KRAS, CDK2, CDK4, MYC, and NPM1 (**Figure 7**). Gene Ontology (GO) and KEGG pathway enrichment analysis indicated that CDKN2A-linked genes were involved in processes such as the G1/S transition, G2/M transition, cellular senescence, Ras protein signaling, and cell division (**Table 1**).

Table 1. Enrichment analysis results for CDKN2A-associated genes

Gene term	Gene count	*P*-value	Genes
Biological processes			
GO:0000082~G1/S transition of mitotic cell cycle	6	6.050814181752417E-11	CDK6, CCND2, CCND1, CDK4, MYC, CDK2
GO:0010389~regulation of G2/M transition of mitotic cell cycle	4	1.871658073473485E-8	CDK6, CDKN2A, CDK4, CDK2
GO:0090398~cellular senescence	4	1.8957134610072728E-6	NPM1, CDKN2A, CDK2, TP53
GO:0007265~Ras protein signal transduction	4	7.304047046494659E-6	CDKN2A, CDK2, KRAS, TP53
GO:0051301~cell division	5	1.663144962206214E-5	CDK6, CCND2, CCND1, CDK4, CDK2
Cellular components			
GO:0000307~cyclin-dependent protein kinase holoenzyme complex	5	2.1409941400482225E-9	CDK6, CCND2, CCND1, CDK4, CDK2
GO:0005813~centrosome	6	3.815763739803654E-6	NPM1, CDK6, CCND2, CCND1, CDK2, TP53
GO:0005737~cytoplasm	10	9.096498278877434E-6	NPM1, CDK6, CCND2, CCND1, CDKN2A, CDK4, MYC, CDK2, KRAS, TP53
GO:0005654~nucleoplasm	9	1.4245291009844775E-5	NPM1, CDK6, CCND2, CCND1, CDKN2A, CDK4, MYC, CDK2, TP53
GO:0005730~nucleolus	6	1.3473568936354612E-4	NPM1, CCND2, CDKN2A, CDK4, MYC, TP53
Molecular function			
GO:0001046~core promoter sequence-specific DNA binding	3	2.3108479185177537E-5	NPM1, MYC, TP53
GO:0016538~cyclin-dependent protein serine/threonine kinase regulator activity	3	7.793826870580279E-5	CCND2, CCND1, CDK4
GO:0004693~cyclin-dependent protein serine/threonine kinase activity	3	7.793826870580279E-5	CDK6, CDK4, CDK2
GO:0030332~cyclin binding	3	1.1405424441090305E-4	CDK6, CDK4, CDK2
GO:0019901~protein kinase binding	4	0.0013063490610775586	NPM1, CCND2, CCND1, CDKN2A
KEGG pathway			
hsa04218:Cellular senescence	9	8.283955501554002E-15	CDK6, CCND2, CCND1, CDKN2A, CDK4, MYC, CDK2, KRAS, TP53
hsa04110:Cell cycle	8	4.022392188086772E-12	CDK6, CCND2, CCND1, CDKN2A, CDK4, MYC, CDK2, TP53
hsa04115:p53 signaling pathway	7	8.407192281962224E-12	CDK6, CCND2, CCND1, CDKN2A, CDK4, CDK2, TP53
hsa05220:Chronic myeloid leukemia	7	9.895313060617844E-12	CDK6, CCND1, CDKN2A, CDK4, MYC, KRAS, TP53
hsa05203:Viral carcinogenesis	8	2.5566041564744064E-11	CDK6, CCND2, CCND1, CDKN2A, CDK4, CDK2, KRAS, TP53

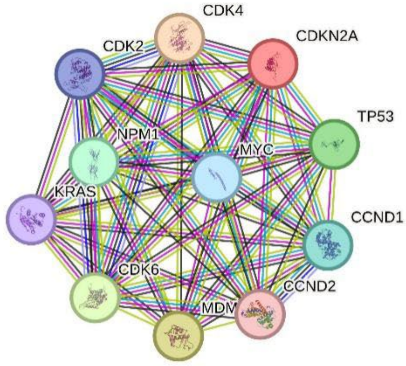

Figure 7. PPI network of CDKN2A using the STRING tool

3.8. Drug sensitivity analysis of CDKN2A

The correlation between CDKN2A mRNA expression and the efficacy of various therapeutic agents was investigated using the Gene Set Cancer Analysis (GSCA) database. The results revealed a strong positive correlation between CDKN2A expression and drug sensitivity to agents such as (5Z)-7-Oxozeaenol, Bleomycin, Cytarabine, Nutlin-3a (-), PD-0332991, and Midostaurin (**Figure 8**). CDKN2A emerged as a critical gene significantly associated with drug sensitivity, especially for drugs with an FDR of less than 0.05, making it a promising therapeutic target for treating COAD, LIHC, and UCEC.

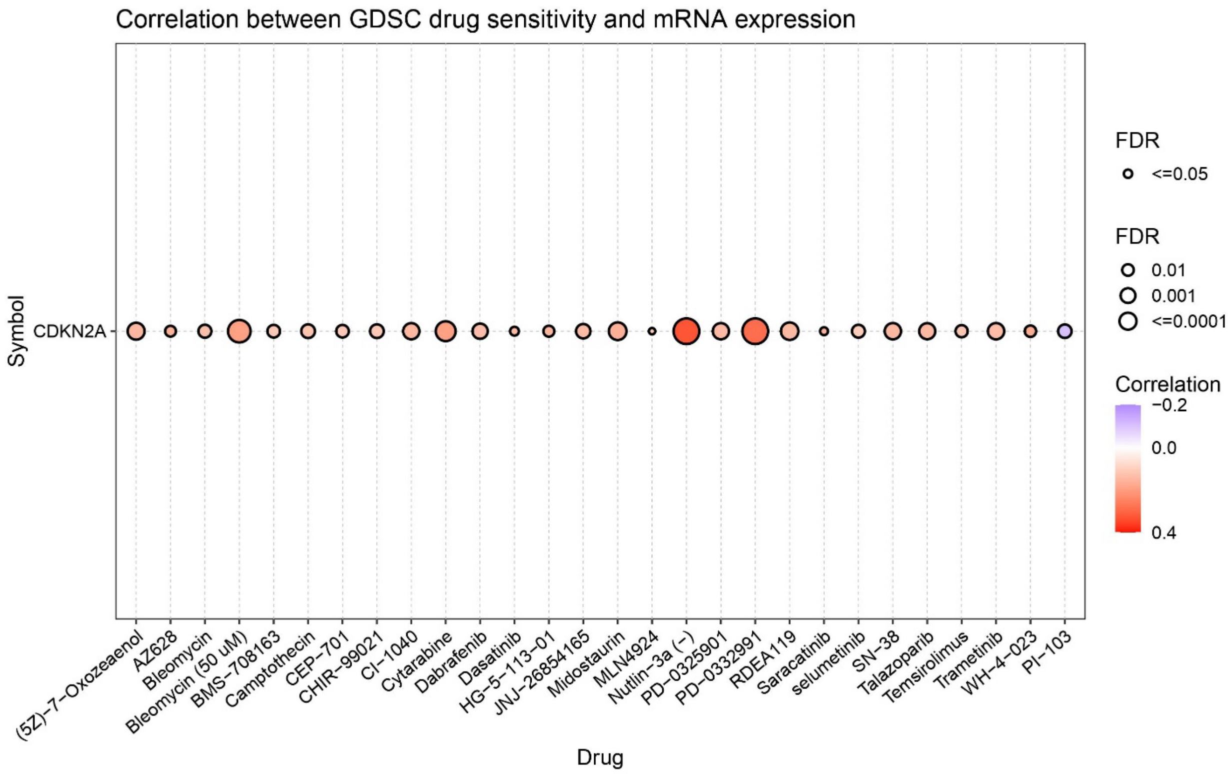

Figure 8. Drug sensitivity analysis using the GSCA database for CDKN2A. Blue indicates a negative correlation, while red indicates a positive correlation. *P-value < 0.05.

4. Discussion

Cancer remains a major global cause of death, posing significant challenges to effective treatment and adversely impacting health [43]. The identification and validation of biomarkers associated with different cancer types are crucial for improving the detection and treatment of malignancies. CDKN2A, located on chromosome 9p21.3, encodes a cyclin-dependent kinase inhibitor and is categorized as a tumor suppressor gene, playing a pivotal role in cell cycle regulation. It encodes two critical proteins, p16INK4a and p14ARF, whose mutations can lead to uncontrolled cell division, contributing to tumor development [44,45]. Mutations in CDKN2A have been linked to various cancers, including melanoma, pancreatic cancer, glioblastoma, bladder cancer, and HNSC [46-49]. Understanding its role in cancer biology not only advances knowledge of carcinogenesis but also opens the door to research into its potential as a diagnostic and prognostic biomarker, as well as a therapeutic target.

The present study demonstrated that CDKN2A was significantly upregulated in the tissues of all 24 major cancer types ($P < 0.05$), including BLCA, ESCA, HNSC, COAD, LIHC, UCEC, and UVM compared to normal control samples. Additionally, the analysis revealed a strong correlation between upregulated CDKN2A expression and lower OS in UCEC, COAD, and LIHC. These findings suggest that CDKN2A plays a critical role in the initiation, development, and progression of UCEC, COAD, and LIHC. Therefore, these three cancer subtypes were the focus of further investigation in this study.

Subsequent analysis of CDKN2A expression across various clinicopathological features, including cancer stages, patient races, genders, and ages in UCEC, COAD, and LIHC, revealed notable overexpression in tumor samples compared to normal controls. Various factors, such as methylation profiles and genetic alterations, have been shown to significantly modulate gene expression [50,51]. Based on this, the present study investigated the genetic mutations and promoter methylation levels of CDKN2A in UCEC, COAD, and LIHC using the OncoDB and cBioPortal databases. The results revealed a positive correlation between CDKN2A expression and promoter methylation, emphasizing the complexity of gene regulation and the involvement of various factors. Furthermore, the analysis of CDKN2A mutations in COAD, LIHC, and UCEC revealed low mutation frequencies of 1.8%, 8%, and 0.8%, respectively. These findings suggest that while hypermethylation may have a significant impact on expression regulation, genetic mutations likely play a minor or negligible role in regulating CDKN2A expression in these cancers.

Immune cell infiltration plays a crucial role in tumor proliferation, metastasis, and invasiveness, influencing clinical outcomes and immunotherapy responses [52,53]. This study explored the relationship between CDKN2A expression and immune cell infiltration in UCEC, COAD, and LIHC using TIMER2.0. The results indicated a negative correlation between CDKN2A expression and the infiltration of $CD8^+$ and $CD4^+$ T cells in UCEC, while no correlation was observed between CDKN2A expression and the infiltration of $CD8^+$ T cells, $CD4^+$ T cells, or B cells in COAD and LIHC. These findings suggest that CDKN2A may have a specific role in immune modulation in UCEC, while its influence on immune cell infiltration in COAD and LIHC appears limited.

The PPI network analysis for CDKN2A identified direct interactions with ten genes and revealed significant enrichment of genes involved in the G1/S transition of the mitotic cell cycle, regulation of the G2/M transition, Ras protein signal transduction, and cell division ($P < 0.05$). The cyclin-dependent protein kinase holoenzyme complex and cyclin-dependent protein serine/threonine kinase regulator activity were among the enriched biological processes, molecular functions, and cellular components, along with KEGG terms related to the cell cycle, signaling pathways, chronic myeloid leukemia, and viral carcinogenesis. These results suggest that CDKN2A may be integrated into multiple pathways, modulating associated genes involved in tumorigenesis. Pathways such as the G1/S transition of the mitotic cell cycle and regulation of the G2/M transition are crucial for genomic replication, growth, and segregation [54-57]. Disruptions in these pathways have also been associated with adverse prognoses in cancer [55,58].

Moreover, analysis using the GSCA database revealed a strong correlation between CDKN2A mRNA expression and drug sensitivity, indicating a positive association with therapeutic agents such as (5Z)-7-Oxozeaenol, Bleomycin, Cytarabine, Nutin-3a (-), PD-0332991, and Midostaurin. These findings suggest that CDKN2A may serve as a predictive biomarker for favorable responses to specific therapies in UCEC, COAD, and LIHC, highlighting its potential as a therapeutic target.

5. Conclusion

This study demonstrated significant upregulation of CDKN2A in UCEC, COAD, and LIHC samples, which was associated with poorer prognoses in these cancers. The correlation between CDKN2A upregulation and the development and progression of UCEC, COAD, and LIHC suggests that CDKN2A may serve as a common diagnostic and prognostic biomarker for these cancer types. However, further research is required to validate these findings and establish their clinical relevance.

Disclosure statement

The authors declare no conflict of interest.

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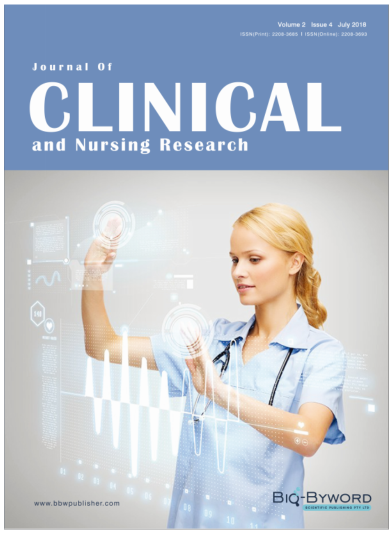

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