Review

Patient-derived tumor models in cancer research: Evaluation of the oncostatic effects of melatonin

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ABSTRACT

The development of new anticancer therapies tends to be very slow. Although their impact on potential candidates is confirmed in preclinical studies, ~95 % of these new therapies are not approved when tested in clinical trials. One of the main reasons for this is the lack of accurate preclinical models. In this context, there are different patient-derived models, which have emerged as a powerful oncological tool: patient-derived xenografts (PDXs), patient-derived organoids (PDOs), and patient-derived cells (PDCs). Although all these models are widely applied, PDXs, which are created by engraftment of patient tumor tissues into mice, is considered more reliable. In fundamental research, the PDX model is used to evaluate drug-sensitive markers and, in clinical practice, to select a personalized therapeutic strategy. Melatonin is of particular importance in the development of innovative cancer treatments due to its oncostatic impact and lack of adverse effects. However, the literature regarding the oncostatic effect of melatonin in patient-derived tumor models is scant. This review aims to describe the important role of patient-derived models in the development of anticancer treatments, focusing, in particular, on PDX models, as well as their use in cancer research. This review also summarizes the existing literature on the anti-tumoral effect of melatonin in patient-derived models in order to propose future anti-neoplastic clinical applications.

1. Introduction

Cancer, which is one of the most significant threats to human life and health worldwide [1,2], accounts for more than 9.5 million deaths and more than 18 million new cases each year [3,4].

However, despite the importance of this disease and the billions of dollars devoted to the screening of innovative anticancer drugs, advances in the development of new therapeutic alternatives have been very slow [5]. One of the main reasons for this has been the failure of drug candidates when tested in clinical trials, with only ~5 % being approved in phase III trials due to the limited efficacy of the drugs tested [6,7]. While the therapeutic effects of potential anticancer agents are adequately confirmed through cell biology and animal models in preclinical studies, the discrepancy between preclinical models and real patients makes it impossible to predict therapeutic efficacy in humans and leads to clinical trial failure [1,8,9]. Thus, the lack of reproducible, accurate and relevant preclinical models for the disease further impedes advances in cancer therapies [10–12].

In addition, during the treatment of clinical tumors, the existing differences between individual patients with similar tumoral manifestations represent a major challenge. The treatment strategy applied is often non-individualized, and existing treatments have different levels of efficacy in patients [13]. Currently, there no efficient methods exist to evaluate a cancer patient's responsiveness to therapeutic drugs. Thus, the effective choice of medical treatment, mediated, in particular, via accurate drug-screening models, is urgently required to enhance

treatment efficacy [5].

With regard to these issues, patient-derived models, which have emerged as a powerful tool in oncology, could overcome some of the problems mentioned above. Several studies highlight the usefulness of these models in testing the therapeutic efficacy of conventional regimens in individualized patients and in identifying new treatment strategies [10,14,15].

These findings demonstrate the value of patient-derived models for investigating standard-of-care anticancer treatment and for discovering novel therapeutic approaches for this deadly disease [10].

2. Limitations of existing preclinical models

Human cancer cell lines established by the US National Cancer Institute, as well as cell line-based mouse xenografts, have been a fundamental tool in cancer research for decades [1,6]. These models have been used to evaluate the effectiveness of potential anticancer compounds given their cost-effectiveness, ease of maintenance, propagation, reproducibility and high-throughput testing capability [16].

However, conclusions reached on the basis of traditional cancer cell lines do not readily apply to patients [1], and the antitumor effects demonstrated using this approach are often not validated in clinical practice [16]. Despite being derived from actual patients, many of these cancer cell lines, established several decades ago, may not accurately represent the primary tumor and differ considerably from the patients' real cancer [10,17].

This discrepancy is explained by several factors. Firstly, the behavior and genetic composition of these cells are modified after thousands of generations in culture [1]. The factors that contribute to genetic alterations, as well as to the phenotypic and morphological characteristics of patients' cancer cells include the composition of cell culture media and plastic supports [18–20]. Specifically, the incorporation of fetal calf serum into the culture medium can trigger cellular differentiation and significant genetic irregularities [13,21,22]. In addition, cell lines cultured in serum-contained media have even been reported to weaken drug resistance mechanisms [13].

Secondly, these cell lines lack a real tumor microenvironment (TME) [1], which, apart from cancer cells, encompasses the adjacent lymphatics, capillaries, stromal cells (immune cells and cancer-associated fibroblasts), other normal cells, the extracellular matrix (ECM), and diverse signaling molecules; all these elements are widely recognized to be crucial factors involved in the progression of cancer [8,23–25]. The absence of immune and stromal cell components in established cancer cell lines impedes to replicate microenvironment features such as tumor-stroma crosstalk, cellular interactions, and three-dimensional tumor niche structures [18,26,27]. As a result, the assessment of drugs, whose mechanism of action is associated with angiogenesis and cell-cell interactions, is impaired [16,28].

Thirdly, long-term culture and selective pressure in vitro promote the survival of clones with specific features, which are selected and outlive other subpopulations [8,16,29]. Consequently, the heterogeneity of tumor subclones is diminished, resulting in the promotion of a more uniform cell population, which fails to accurately represent the heterogeneity of existing tumors [18]. Finally, most of these cell lines have been reported to be contaminated with other cells [1]. Accordingly, some studies suggest that cell lines derived from diverse tumors exhibit greater similarity to each other than to the corresponding tumors from which they originated [13,30].

All these limitations lead to unreliable results based on cell line-derived models. Subsequently, translational medicine institutions and pharmaceutical companies are no longer satisfied with the results obtained from classical cancer cell line-derived models alone [1] and have prevented the use of these models for drug screening and evaluating preclinical drug efficacy [31,32].

Given all the above, the development of accurate evaluation models similar to those used in clinical cancer research practice is urgently required [1].

3. Patient-derived models

The considerable discrepancy between preclinical evaluation models and clinical practice has encouraged the adoption of innovative technologies [1,33]. In this context, the preservation of tumor heterogeneity, high genomic and transcriptomic fidelity and the presence of an accurate TME are some of the main elements that need to be implemented in drug testing tools in order to obtain results that can be transposed into clinical practice [16,31,34].

The concept of patient-derived models has emerged and received broad acceptance in cancer research platforms [1]. Patient-derived platforms have enabled researchers to perform drug testing evaluations in models derived from patients [16,31]. Currently, there are three patient-derived models: patient-derived xenografts (PDXs), patient-derived organoids (PDOs), and patient-derived cells (PDCs) (Fig. 1) [1].

3.1. Patient-derived xenografts (PDXs)

PDX models are generated by the implantation of human tumor tissues or cells into immunodeficient mice (Fig. 1) [5,8,35]. Unlike cell line-derived tumor models, PDXs are distinguished by their maintenance of the cellular and molecular heterogeneity of the patient's original tumor [5,16]. In addition, these models are histologically and genetically closer to the primary tumor from which they originated [8,36,37].

Consequently, PDXs are crucial for enhancing our understanding of the genetic and molecular etiology of cancer [8,38,39]. In addition, the PDX model, which is recognized as the perfect model for anticancer drug evaluation, can accurately reproduce the treatment response of the original tumors and provide information that can support the selection of therapeutic regimens [5,40–44]. Nevertheless, the PDX model still faces several obstacles, including its high cost, time-consuming nature, unsatisfactory success rate and limited efficiency in throughput screening [1,45].

3.2. Patient-derived organoids (PDOs)

Three-dimensional patient-derived tumor models, more commonly known as tumor organoids or tumoroids, have also shown their value in the study of cancer biology and in performing drug screenings [46–48]. Over the last decade, PDO models of several cancer types, including cancer of the intestinal tract, breast, prostate, lung, kidney, liver, head and neck, and bladder, have been described [49–53].

To generate PDOs, patient-derived tumor tissue is firstly digested into single cells or clusters and then transplanted to a basement membrane extract with a specific growth medium (Fig. 1) [1,54–56], thus creating models that maintain aspects of the tumor structure and heterogeneity [3,57–59].

Compared to more conventional two-dimensional cancer cell line cultures, PDOs are able to mimic the TME, to study tumor and immune system interactions and to easily model and adapt tumor genetics [49, 60,61]. In addition, although the PDX model, which is implemented in vivo, is often preferred over the PDO model, PDO has two important advantages since PDOs can be used for immunity investigations, and all grades of tumors can theoretically be used to establish a PDO [1,62]. These PDOs, which can mimic the histology and genetic make-up of the parental tumor, can facilitate preclinical and pharmacological studies [49,63,64], thus indicating their impressive predictive abilities [3,42, 65–67]. However, like PDXs, the establishment of a PDO model can also be time-consuming, costly, and technically difficult [1].

3.3. Patient-derived cells (PDCs)

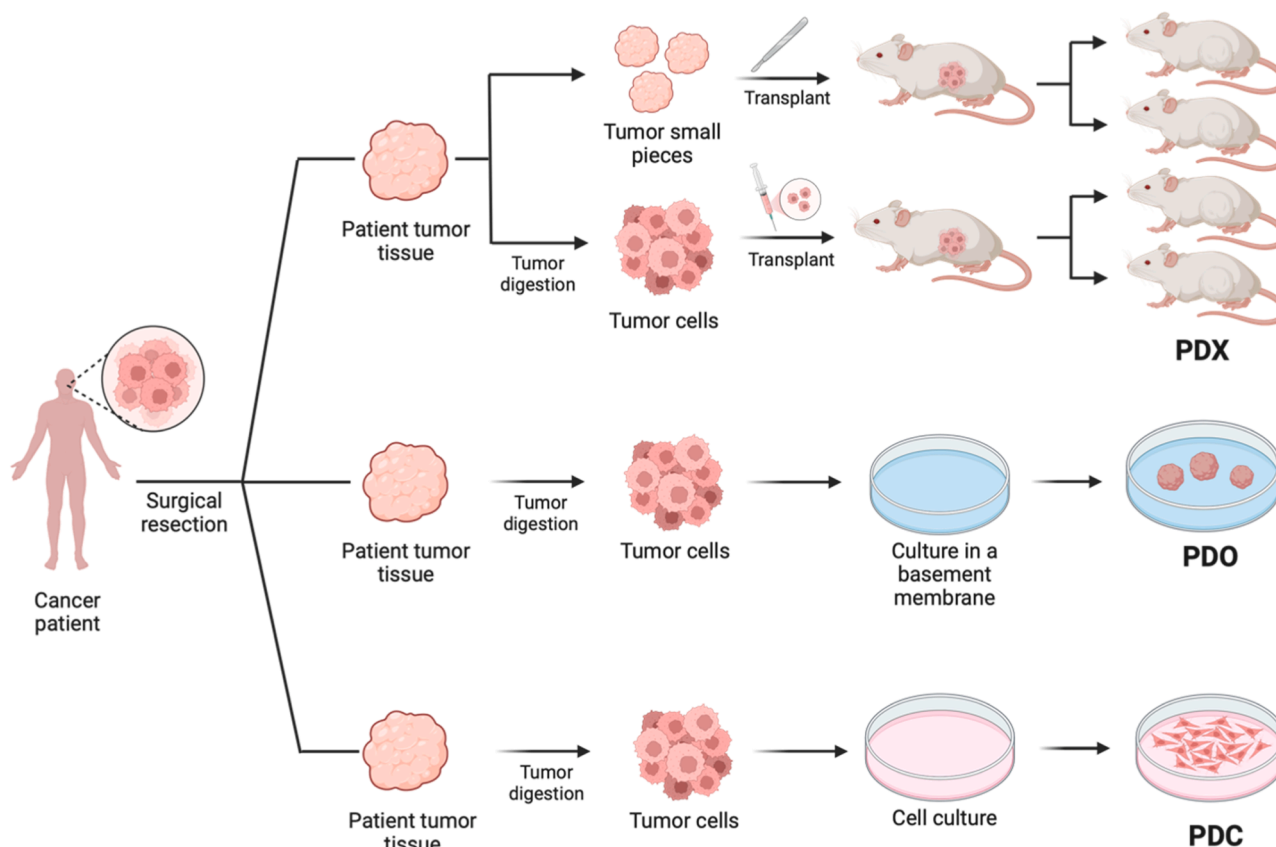Despite being the most faithful representations of the human body

Fig. 1. Schematic representation of the process followed to establish the three different patient-derived models. All of them were derived from the actual patient's tumor tissue, but using the different approaches: patient-derived xenografts (PDXs); patient-derived organoids (PDOs); and patient-derived cells (PDCs). The image was created using Biorender.com (accessed on 28 August 2023).

and disease, PDXs and PDOs are characterized by high costs and time-intensive processes, which ultimately restrict their rate of utilization in cancer research. Given the limitations of PDXs and PDOs and that traditional cancer cell lines are no longer a credible model, the PDC model appears to be an ideal substitute for classical cancer cell lines. This model involves the use of cells directly digested and derived from the patient's tumor tissue (Fig. 1) [1,68].

However, like traditional cancer cell lines, controlling the source and quality of a PDC model can be challenging, and it is difficult to replicate the experimental results among different PDCs. Furthermore, the divergence between PDC models and the complexities of the human body also undermine the authenticity of the research data. Nevertheless, PDC remains an essential patient-derived model for cancer research, especially in the early stages of research and even in the screening of drug candidates [1,69–71].

4. PDX model in cancer research

At present, all patient-derived models are widely applied in various areas of medicine, including fundamental research, drug development, and clinical applications, and will continue to contribute to the development of anticancer treatments in the future. However, while all these models are derived from patients, because the PDX model is implemented in vivo, it is considered to be more accurate and reliable as compared to models implemented in vitro [1]. As a result, this review will focus on the PDX model and its application in cancer research.

4.1. PDX establishment variables

As mentioned above, PDX models are obtained by implanting tumor

tissues or cells into immunodeficient mice [8,72,73]. The success rate of PDX establishment depends on multiple variables including cancer type, the technique used to implant the tumor and the animal recipient [16, 74,75].

On the one hand, the success rate of tumor transplantation has been found to be higher in patients with tumors exhibiting high malignancy and low differentiation [13,75,76]. Moreover, specific tumor types, such as colorectal or gastric cancer, have demonstrated a higher probability of engraftment as compared to malignancies originating from other sites such as the breast or kidney [16].

On the other hand, many techniques have been employed to optimize PDX engraftment. Firstly, tumor sample collection and storage play a pivotal role in PDX development. It is recommended to minimize the time between sample collection and implantation, as well as to shorten the duration of the process of implanting the tumor into mice. In addition, the implantation of solid tumor fragments has had a higher success rate than the implantation of a suspension of single cancer cells after tumor dissociation. This may be attributed to the preservation of tumor architecture, which might facilitate a successful and faster engraftment [6,77–79].

The rate of tumor engraftment also depends on the implantation site. In orthotopic transplants, tumor samples are implanted into the same anatomical site as the patient tumor [6]. This method, which usually increases the PDX engraftment success rate, provides a tumor growth microenvironment that more closely resembles the microenvironment in patients, which is conducive to tumor occurrence [80]. However, this transplantation method is technically more challenging and cannot always be performed [8,81]. Moreover, in the case of hormone-dependent cancers, it has been reported that the addition of human hormones could facilitate tumor engraftment [16].

Finally, with regard to the animal recipient, the probability of obtaining successful engraftment increases with the degree of immunosuppression in the animal host [16,82,83]. Initially, PDXs were performed using athymic nude mice as hosts, although these murine models showed low engraftment rates [18]. This murine strain has the least compromised immune system with natural killer (NK) cells present, which often contributes to tumor xenograft rejection [6].

A higher rate of success can be achieved using animal models lacking the functions of both B and T lymphocytes and of NK cells such as non-obese diabetic (NOD)/severe combined immunodeficient (SCID) mice, particularly, NOD/SCID/IL-2 receptor-g deficient (NOG and NSG) and NOD/SCID/Janus kinase 3 deficient (NOJ) mouse models [16,84,85]. The use of these strains with higher immunodeficiency could raise engraftment efficacy to an 80 % success rate [16,86].

Furthermore, while mice are the predominant hosts for PDX generation, other species, such as zebrafish, can also be utilized for this purpose. Zebrafish, a non-mammalian species, offer certain advantages over traditional mouse models. These advantages include a higher breeding rate, lower maintenance costs, and the ability to track malignant cells using fluorescent labeling of the transparent Casper zebrafish strain. Moreover, the engraftment process for zebrafish PDXs is easier and faster as compared to their murine counterparts [16,87–90].

Given all these variables, PDXs can be successfully established, and also enable the evaluation of cancer biology and treatment strategies in a complex organism [16].

4.2. Applications of the PDX model

Once successfully established, PDXs are critical to improving our understanding of the genetic and molecular etiology of cancer, as well as to developing and validating effective therapies [8,91–94].

These models are applied in different areas. Firstly, PDXs have played a valuable role in fundamental research for many years. Superior to traditional cell lines, PDXs can simulate the heterogeneous and complex tumor microenvironment of actual patients, which is used to reevaluate the cancer biomarkers screened by traditional technologies [1,95–97]. Moreover, given that the majority of PDXs exhibit the same histopathological and molecular features as the primary tumors, these models are gaining attention in the area of drug development [8,98,99]. The PDX model, which is regarded as the pre-experiment of phase II clinical trials, is sometimes called "clinical trial phase 0". Before approval in clinical trials, the efficacy of innovative anticancer drugs is evaluated and validated by the PDX model. PDXs are also an appropriate tool to discover drug-sensitive markers and to screen drug combination strategies [1,100–105].

On the other hand, in recent years, the PDX model has been widely used in clinical practice [106–108]. Directly derived from the tumor tissue, PDXs, which can be used to screen an effective clinical treatment for a specific patient, may be the most reproducible and homologous model of the disease. As previously mentioned, inherent heterogeneity among cancer patients often results in different responses to the same therapy. Hence, a personalized therapeutic strategy, also called personalized treatment, is crucial in clinical practice [109–111]. However, evaluating hundreds of drug candidates independently and determining the ideal strategy to be adopted is impractical. Fortunately, PDX models have emerged as a viable alternative for patients themselves in order to identify the optimal treatment strategy. Existing studies indicate an overall predictive accuracy of 90 % for PDX models, thus highlighting their efficacy to design effective personalized treatment in a clinical context [1,112,113].

With regard to personalized treatment, a valuable new tool, known as the mini-PDX, has been developed to enable clinicians to selection the appropriate therapy, especially chemotherapeutic agents. Mini-PDX is a drug sensitivity test model that retains the oncogenicity of patients' tumor cells. In this model, cells human tumor tissues are encased in special capsules and injected into immunocompromised mice in order to create tumor xenografts. This promising model reduces the complexity of the process and obtains faster results as compared to traditional PDX models. The mini-PDX model requires only a small number of tumor cells and rapidly assesses drug sensitivity in an average testing time of 7 days, enabling patients to receive personalized treatment within a clinically relevant time frame (Fig. 2). Previous studies have demonstrated a strong consistency between mini-PDX- and PDX-based drug sensitivity predictions for a variety of solid tumors, indicating that the mini-PDX-based drug sensitivity model can accurately predict the therapy outcome of cancer patients with cancer [5,114].

Briefly, PDX could be the ideal model to simulate actual human disease for cancer research. Consequently, in research institutions, pharmaceutical companies, and medical organizations, the PDX model is widely used to identify biomarkers, as well as to screen clinical drugs and precision treatments for different cancers [1,8].

4.3. Limitations and challenges of the PDX model

Despite all the advantages of PDX model, there remain several challenges that limit its broad use in clinical practice [1,5,115].

Firstly, the PDX model has a low success rate. Highly malignant tumors have a better engraftment rate although low grade malignancies have also been used for PDX modelling. Additionally, PDX requires a large quantity of tumor content. Therefore, minimization of fat and connective tissue in the fragments to be implanted is very important. These fragments with high tumor content can only be obtained from tumor removed biopsies, which limits its application to a limited number of cancer patients [1,5,116].

Secondly, PDX modelling can be a time-consuming process. Firstly, the use of PDX requires a rigorous and time-consuming ethical approval process. Secondly, the establishment of a PDX model often requires several months to carry out and involves substantial costs, which is a great drawback for both researchers and patients [1]. According to existing studies, it takes approximately 4–8 months to assess the treatment efficacy of PDX models for a specific cancer. The time lag between the transplantation of tumor tissue to mice and the initiation of treatment is a limiting factor, which restricts its broader application [5]. Among patients with rapid tumor development and a short expected survival rate, some may succumb to the disease before obtaining the results of the drug sensitivity tests [13,117]. This challenge has been overcome by the development of the mini-PDX model, which, as mentioned above, is much faster than traditional PDXs [5].

Thirdly, it is difficult to conduct studies on cancer immunity in the PDX model, as immune deficiency is indispensable for this type of modelling [1,82]. Therefore, the use of PDX models to evaluate drugs targeting immune-mediated anti-tumor efficacy is hindered. To overcome this, humanized mouse PDX models have emerged as a promising alternate [8,118,119]. These models can be obtained by xeno-transplanting human immune cells into irradiated mice or by engineering the host to express specific human genes [16,120]. Humanized mice and mice with reconstituted human immune systems are currently under investigation and should facilitate the testing of different anti-cancer strategies, including immunotherapy within the PDX model [1, 120–124].

Finally, most PDX models involve subcutaneous transplants, which are often quite different from the primary environment of tumors where few metastases occur. Accordingly, PDXs cannot often be used as a metastasis model. Orthotopic transplantation of PDX models can effectively solve this problem. Patient-derived orthotopic xenografts (PDOXs) provide a tumor growth microenvironment that more closely resembles the real situation in patients, which is conducive to tumor occurrence and metastasis. However, in contrast to subcutaneous transplants, which are easier to manipulate, PDOXs require highly technical skills and resources [8]. Tumor inoculation and tumor growth monitoring of orthotopic xenografts are technically more difficult and invasive than subcutaneous models [6]. Consequently, the methods
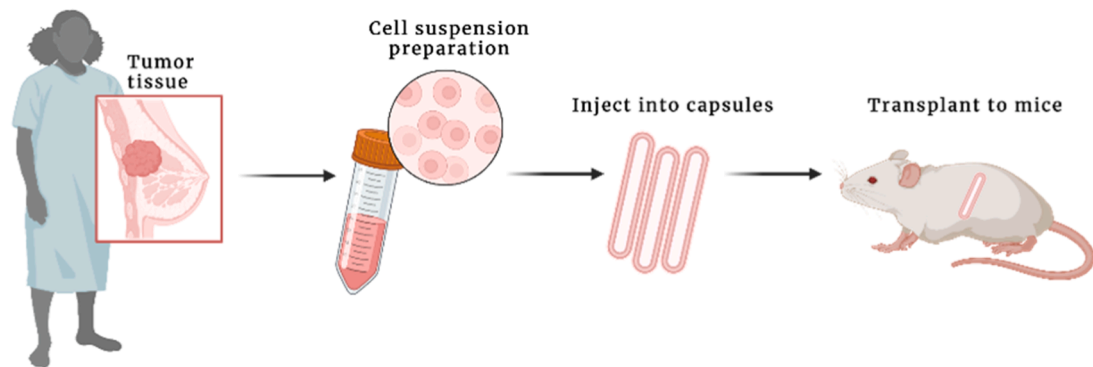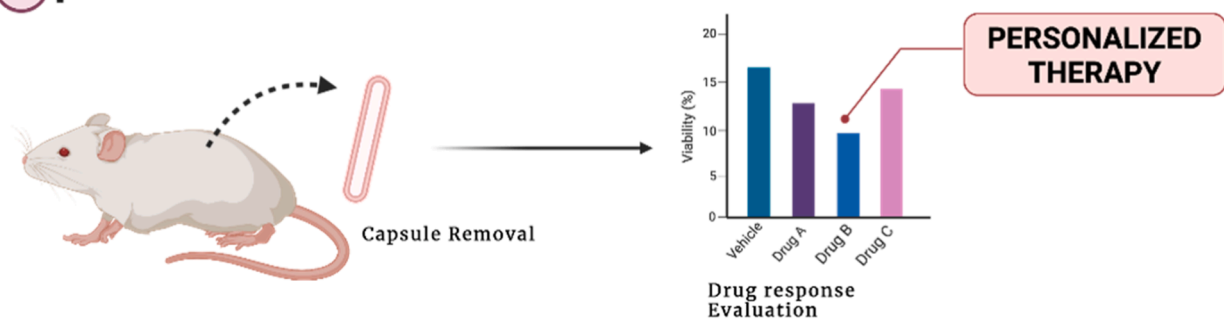
Fig. 2. Schematic representation of 7-day mini-PDX process. Imaged using Biorender.com (Accessed on 28 June 2023).

utilized to assess tumor formation in deep organs need to be improved [13].

In conclusion, although the PDX model has a bright future in cancer research, it is still faced with several obstacles that impede its broader application. Nevertheless, most of these challenges are expected to be overcome through future scientific and technological advances which should expand the scope of the PDX model [1].

5. Oncostatic activities of melatonin

Directly derived from actual patients, the results obtained from patient-derived models, especially PDXs, are factual, reliable, and effective. Consequently, these models have opened up the possibility of developing innovative anticancer therapies that are properly applied in clinical practice [1]. Because of its oncostatic impact and lack of association with adverse effects, melatonin (N-acetyl-5–methoxytrypt-amine) is of particular relevance to the development of innovative cancer treatments [125]. Even though the use of patient-derived models has significantly increased in the last 5 years, little is known about the oncostatic effect of melatonin in patient-derived tumor models.

Over recent decades, accumulating evidence has outlined the

relevance of melatonin to human physiology and pathology. Numerous studies have supported the anticancer properties of melatonin [126–128] in breast, ovary, prostate, skin, liver and head and neck cancer. [129,130].

Anticancer function of melatonin involves multiple mechanisms: direct pro-apoptotic actions, decreasing the uptake of growth factors involved in tumor growth signaling pathways, suppressing cell cycle progression, increasing immunosurveillance and anti-angiogenic or anti-metastatic effects [129]. Moreover, melatonin can also promote reactive oxygen species (ROS) generation leading to cell death in a variety of cancers [125–127]. However, melatonin, together with its metabolites, are potent free radical scavengers and broad-spectrum antioxidants with evolutionarily conserved properties in normal cells. Therefore, melatonin can act as an antioxidant in normal cells and as a pro-oxidant in cancer cells [131]. These pro-oxidant actions in tumor cells are responsible for most of their anti-tumoral effects, as previously demonstrated in numerous studies [125–127].

5.1. Clinical trials based on the anticancer effects of melatonin

The properties of melatonin observed in both tumoral and normal

tissue suggest that melatonin treatment is remarkably effective in terms of its clinical translation to cancer patients [132].

Nevertheless, although melatonin's antineoplastic activity has been widely explored in both in vivo and in vitro models of carcinogenesis [133], when applied in clinical trial studies, some conflicting results have arisen. Some of the most important clinical trials regarding melatonin's oncostatic effects demonstrate positive impacts on the anticancer treatment to alleviate the chemotherapy-related side effects without any significant effectiveness in cancer cells themselves.

In a randomized double-blind clinical study, melatonin was co-administered to patients with HNSCC. The results show that the administration of melatonin reduced mucositis, one of the main side effects of radiation in HNSCC patients, and also ameliorated pain [134, 135]. In addition, combining melatonin with a standard CDDP-based standard treatment of this type of cancer reduced anemia, a common side effect of cisplatin [136]. The amelioration by melatonin of the side effects of chemotherapy was also investigated in patients with gastrointestinal cancer, which showed that, though capable of maintaining body weight, melatonin failed to attenuate cachexia [137]; on the other hand, metastatic colorectal patients, who received a combined melatonin/subcutaneous IL-2 treatment following first-line 5-FU therapy, showed a higher survival rate after one year as compared to those who only received 5-FU treatment [138]. Similarly, a randomized clinical trial showed that metastatic breast cancer patients treated with both tamoxifen and melatonin had a higher relative response rate as compared to those receiving tamoxifen alone [139].

Although an investigation of the use of melatonin in non-small cell lung cancer (NSCLC) patients has also shown that melatonin improves their quality of life, it did not demonstrate any protection against chemotherapy-related side effects [140]. In addition, while treatment with adjuvant melatonin following NSCLC resection increased the 2-year disease-free survival rate of patients with late-stage disease, it did not beneficially affect their quality of life, symptoms, or immune function [141].

In summary then, most of the results of these clinical trials corroborate melatonin's protection against the side effects of chemotherapy or radiotherapy [134,135]. In general, treatment with melatonin enhances the efficacy of therapies, alleviates treatment-related side effects and improves patients' quality of life, including a reduction in the incidence of depressive symptoms and an improvement in the quality of sleep of cancer patients [142,143]. However, melatonin's oncostatic properties have not always been demonstrated in clinical trials [134,139], which, as mentioned above, could be explained by the type of evaluation model used in the experiments.

5.2. Evaluation of the oncostatic effect of melatonin in patient-derived tumor models

The oncostatic effect of melatonin in patient-derived tumor models has been little studied. Only five published studies have evaluated the role of melatonin in patient-derived tumor models and only three deal with PDX models (Table 1) [144–148].

The antitumor effects of melatonin were evaluated in tumor organoids derived from colorectal cancer (CRC) patients' tumors in two different studies [144,147]. Sakatani et al. [144] demonstrated that 2 mM melatonin significantly inhibited the growth of patient-derived CRC organoids. In line with these results, Sharda et al. [147] also demonstrated that 0.5 mM melatonin, alone or combined with andrographolide, reduced CRC organoid growth. Both studies highlight the inhibitory growth potential of melatonin.

In addition, the oncostatic effects of melatonin have been studied in two different PDX models, derived from breast [145] and oral tumors [146]. Hasan et al. [145] established a PDX model from a mastectomy specimen of an African American woman to analyze the anti-tumor effect of different melatonin-tamoxifen conjugates. The PDX tumor was propagated and maintained in SCID/Beige immunodeficient mice. After

Table 1
Studies of the oncostatic effect of melatonin in patient-derived models.

Type of cancer	Type of patient-derived model used	Melatonin dosage	Effect	Author
Colorectal cancer (CRC)	PDO	2 mM	Inhibition of colorectal cancer (CRC) organoid growth	Sakatani et al. [144]
Colorectal cancer (CRC)	PDO	0.5 mM	Inhibition of CRC organoid growth	Sharda et al. [147]
Breast cancer	PDX (ex-vivo treatment)	10 µM melatonin-tamoxifen drug conjugate	Decreased cell viability and cell migration	Hasan et al. [145]
Oral cancer	PDX	Intraperitoneal injections of melatonin at 20 mg/kg/ daily for 24 or 42 days	50–80 % inhibition of tumor growth	Yang et al. [146]
Head and neck	PDX	Intratumoral injections of 3 % melatonin every 24 h for 28 days	30–80 % inhibition of tumor growth	Martinez-Ruiz et al. [148]

two serial transplantation passages in mice, the tumor was removed, and small tumor pieces were dissected. However, in this study, the effects of melatonin-tamoxifen drug conjugates were evaluated in these dissected tumor pieces and not directly in mice-bearing breast xenografts. Despite this limitation, in this study, novel melatonin-tamoxifen drug conjugates exhibiting anticancer actions against breast cancer were identified. Two of these melatonin-tamoxifen drug conjugates inhibited cell viability and decreased cell migration in a wound-healing assay. Based on this study, which analyzed the effects of melatonin in patient-derived tumor models, it was possible to confirm that melatonin is a promising candidate for combinatory use with conventional chemotherapeutics for breast cancer treatment.

On the other hand, Yang et al. [146] successfully established two oral cancer PDXs, which revealed a histology consistent with the original clinical cancer tissues. Tumor specimens were obtained from oral squamous cell carcinoma patients with lymphatic metastases during initial surgery. The tumors obtained were subcutaneously implanted into NSG mice. When tumor volume reached approximately 3000 mm^3, the tumor was removed for serial transplantation. When tumor volume reached 500 mm^3 in the fourth or seventh generation of PDXs, depending on the tumor sample, mice bearing PDXs were randomly divided into different experimental groups. Mice were treated with 20 mg/kg/daily melatonin or vehicle (PBS) through intraperitoneal injection for 24 or 42 days. Melatonin was observed to significantly inhibit tumor growth by 50–75 % as compared to the vehicle in both oral cancer PDX models. Melatonin treatment also repressed lysine-specific demethylase (LSD) expression in oral PDX, which has been demonstrated to contribute to tumor survival, growth and metastasis. No apparent toxicity or weight loss in the mice was observed after melatonin administration during the experimental period. In conclusion, the effect of melatonin in oral cancer was demonstrated to be accompanied by LSD1 downregulation in a preclinical PDX model.

Finally, our research group recently established 3 different PDX models of head and neck cancer [148]. Primary tumors were obtained from medically indicated surgeries from head and neck cancer patients. After tumor digestion, the purified cells/cell aggregates obtained were subcutaneously injected into the flank of NSG mice. Once the tumors reached 800–1000 mm^3, the mice were sacrificed and the tumor was extracted, digested and re-implanted into a new generation of 3–5 mice. After 3 generations of mice, the mice bearing PDX were randomly divided into two experimental groups. The mice were treated with

vehicle solution or 3 % melatonin for 28 days. Melatonin or vehicle solution was injected intratumorally every 24 h. Melatonin significantly reduced tumor growth as compared to the vehicle group in the three PDX models established. Tumor growth inhibition varied by 35–80 %, depending on the sample used for PDX establishment. In one of the PDX established, melatonin not only exhibited oncostatic but also antitumoral effects, as the tumors showed complete regression after 28 days of melatonin treatment [148]. These results indicate that the treatment with melatonin should be considered a potential therapeutic strategy for head and neck cancer therapy.

6. Conclusion and perspectives

Although established cell line–based research provides an important insight into the effects of drugs on cancer cells and into their basic mechanisms of action, these findings fail to translate to clinical practice [145]. However, patient-derived tumor models, which can accurately reflect patients' tumors, have transformed the field of drug research. These models have been gaining attention in recent years and are widely applied in various areas of medicine, particularly in translational medicine and personalized treatment. In this context, patient-derived tumor models, which are an appropriate tool for drug evaluation, could fill the gap between basic research and clinical practice [1,149].

On the other hand, the antitumoral effects of melatonin have been widely demonstrated in different studies and cancer models. However, little is known about the oncostatic effect of melatonin in patient-derived tumor models. The lack of research on the effects of melatonin in patient-derived tumor models could explain the inconsistent results regarding melatonin when used as an anticancer therapy in clinical trials. Patient-derived tumor models might better retain the heterogeneity and molecular characteristics of patients´ tumors. Therefore, the use of these models as drug testing tools could provide more accurate results than those derived from established cell line-based models, providing better approaches to be evaluated in clinical trials.

Given all the above, the effects of a melatonin treatment specifically developed for the purposes of cancer therapy need to be evaluated not only in established cancer cell line models but also in patient-derived models in order to ensure translatability to clinical applications. Taking into account the oncostatic properties of melatonin and its lack of toxicity in normal cells, the evaluation of future formulations of melatonin in patient-derived tumor models could lead to the development of an effective and accurate melatonin-based anticancer therapy.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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