Eskisehir Medical Journal
Eskişehir Tıp Dergisi

Eskisehir Med J. 2025; 6(3): 212-219
doi: 10.48176/esmj.2025.207

Original Article / Araştırma Makalesi

DOES RETINOIC ACID PAVE A WAY FROM TESTICULAR EMBRYONAL CARCINOMA TO NEURON?

RETİNOİK ASİT TESTİS EMBRİYONEL KARSİNOMUNDAN NÖRONA BİR GEÇİŞ YOLU AÇAR MI?

HİLAL NAKKAŞ[1], ŞEYMA KİPEL[1]

[1] Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Histology and Embryology, Ankara, Türkiye

ABSTRACT

Introduction: One kind of testicular cancer which affects germ cells that eventually give rise to sperm is called testicular embryonal carcinoma. These cancer cells can self-renew and differentiate, and they encourage the growth of malignancies. An active byproduct of vitamin A (retinol), retinoic acid (RA), is crucial for both embryonic development and fundamental biological functions such cell division, proliferation, and death. It is frequently utilized as a differentiation inducer in vitro, particularly on cancer and stem cells. S100B protein plays an important role in events such as inflammation, cell growth, cell differentiation, cytoskeleton dynamics and cell movement. NF-κB (nuclear factor kappa B) is a protein complex that is an important transcription factor within the cell. Constantly active NF-κB increases cell proliferation in some cancers and inhibits the immune system's response to the tumor. The aim of the study is to demonstrate how the morphology and S100B and NF-κB expressions of testicular embryonal carcinoma cells change as a result of RA stimulation.

Method: After RA was administered to the testicular embryonal carcinoma cells (CRL-2073) at the determined dose (10 μM), crystal violet and luxol fast blue stainings were performed for morphological examination. Then, using immunohistochemical technique, cellular expression and location of S100B and NF-κB in testicular embryonal carcinoma cells were examined.

Results: When we stained CRL-2073 cells differentiated with RA with crystal violet, we observed morphological differences in the cell nucleus and cytoplasm, which is a visual indicator of differentiation. Luxol fast blue staining was observed in CRL-2073 cells that began neuronal differentiation with RA. The S100B protein was expressed in embryonal carcinoma cells and was associated with cell differentiation. NF-κB was active in maintaining proliferation and pluripotency in these cells; its activity decreased with differentiation.

Conclusion: It is important to show histologically that CRL-2073 cells begin to differentiate with RA. In addition, determining the expression levels of S100B and NF-κB proteins, which are biomarkers for both tumor biology and differentiation processes, by immunostaining before and after RA is a potential treatment target. This information is an important step in understanding cancer biology.

Keywords: Testis embryonal carcinoma, retinoic acid, differantiation, S100B, NF-κB.

ÖZET

Giriş: Testis embriyonal karsinomu, sperm oluşumuna yol açan germ hücrelerini etkileyen testis kanseri türlerinden birisidir. Bu kanser hücreleri kendini yenileyebilir, farklılaşabilir ve kötü huylu tümörlerin büyümesini teşvik eder. A vitamininin (retinol) aktif bir yan ürünü olan retinoik asit (RA), hem embriyonik gelişim hem de hücre bölünmesi, çoğalması ve ölümü gibi temel biyolojik işlevler için önemlidir. Özellikle kanser ve kök hücrelerde, in vitro farklılaşma indükleyicisi olarak sıklıkla kullanılır. S100B proteini, iltihaplanma, hücre büyümesi, hücre farklılaşması, hücre iskeleti dinamikleri ve hücre hareketi gibi olaylarda önemli rol oynar. NF-κB (nükleer faktör kappa B), hücre içinde önemli bir transkripsiyon faktörü olan bir protein kompleksidir. Sürekli aktif olan NF-κB, bazı kanserlerde hücre çoğalmasını artırır ve bağışıklık sisteminin tümöre verdiği yanıtı engeller. Çalışmanın amacı, RA uyarımı sonucu testis embriyonal karsinom hücrelerinin morfolojileri ile S100B ve NF-κB ekspresyonlarının nasıl değiştiğini göstermektir.

Yöntem: Testis embriyonal karsinom hücrelerine (CRL-2073) belirlenen dozda (10 μM) RA uygulandıktan sonra, morfolojik inceleme için kristal viyole ve luxol fast blue boyamaları yapıldı. Daha sonra, immünohistokimyasal teknik kullanılarak, testis embriyonal karsinom hücrelerinde S100B ve NF-κB'nin hücresel ekspresyonu ve yeri incelendi.

Bulgular: RA ile farklılaşan CRL-2073 hücrelerini kristal viyole ile boyadığımızda, hücre çekirdeğinde ve sitoplazmada farklılaşmanın görsel bir göstergesi olan morfolojik farklılıklar gözlemledik. RA ile nöronal farklılaşmaya başlayan CRL-2073 hücrelerinde luxol fast blue boyaması gözlendi. S100B proteini embriyonal karsinom hücrelerinde ifade edildi ve hücre farklılaşmasıyla ilişkili bulundu. NF-κB bu hücrelerde proliferasyon ve pluripotensiyi korumada aktiftir; aktivitesi farklılaşmayla azaldı.

Sonuç: CRL-2073 hücrelerinin RA ile farklılaşmaya başladığını histolojik olarak göstermek önemlidir. Ayrıca, hem tümör biyolojisi hem de farklılaşma süreçleri için biyobelirteç olan S100B ve NF-κB proteinlerinin ifade düzeylerinin RA öncesi ve sonrasında immünboyama ile belirlenmesi potansiyel bir tedavi hedefidir. Bu bilgi kanser biyolojisini anlamada önemli bir adımdır.

Anahtar kelimeler: Testis embriyonal karsinom, retinoik asit, farklılaşma, S100B, NF-κB.

INTRODUCTION

Human testicular embryonal carcinoma cells are a type of malignant germ cell tumor cells that occur in the testicles. These cells arise from a particularly aggressive and fast growing type of tumor called embryonal carcinoma. These tumors consist of undifferentiated and rapidly dividing cells similar to cells of embryonic origin (1). They can be very invasive and they can become pluripotent and transform into other cell types. Embryonal carcinoma cells are used as a model system in cancer biology, pluripotent stem cell studies and drug development. Embryonal carcinoma cell lines such as CRL-2073 are frequently used in laboratory settings, especially in studies on differentiation. Human testicular embryonal carcinoma cells show quite characteristic morphological and biological features in cell culture (2). Due

Corresponding Author: Şeyma Kipel, Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Histology and Embryology, Ankara, Türkiye.
E-mail: seymakipel@gmail.com
ORCID: 0000-0002-4176-5136

Submission Date: 11.06.2025 **Acception Date:** 25.07.2025
Cite as: Nakkas H, Kipel S. Does Retinoic Acid Pave A Way From Testicular Embryonal Carcinoma To Neuron? Eskisehir Med J. 2025; 6(3): 212-219. doi: 10.48176/esmj.2025.207.

to their invasive properties, the cells grow in adherent colonies. The colony structure is similar to embryonic stem cells. They proliferate rapidly. They usually divide in culture for 24–36 hours. This high proliferation is an indication that the cells are of tumor cell origin. These cells are pluripotent, meaning they can differentiate into different cell types (ectoderm, endoderm, mesoderm origin) under appropriate conditions (3,4). Therefore, they are the preferred model cells in differentiation studies.

Retinoic acid (RA) is an active derivative of vitamin A (retinol) and plays an important role in embryonic development and basic biological processes such as cell differentiation, proliferation and apoptosis. It is widely used in vitro as a differentiation inducer, especially on stem cells and cancer cells (5). It transforms stem cells or pluripotent cells into specialized cell types. For example, it can direct embryonal carcinoma cells into neuronal cells or other cell types (6). It is critical for embryonic development, especially for the formation of the anterior-posterior axis and nervous system development. It can also cause developmental disorders depending on the dose. Retinoic acid binds to RAR/RXR receptors in the nucleus and acts as a transcription factor, increasing or repressing the expression of target genes (7).

The S100 protein family is a very large and functional group of proteins that play very important roles especially in the connective tissue, nervous system, immune system and cancer biology (8). S100 gets its name from the phrase "solubility in 100% ammonium sulfate". It is a family of small, calcium-binding proteins. It is usually found in cells such as glial cells, Schwann cells, melanocytes, cartilage cells, macrophages, Langerhans cells (9). S100B proteins are proteins directly related to neuronal differentiation and nervous system development. These proteins are not only a tumor marker but also an active biological signal regulator. In in vitro studies, S100B expression is increased during neuronal differentiation with RA at embryonal cells such as NT2 and P19 (10,11). This indicates maturation of cells leading to glial or neuronal pathways. CRL-2073 cells do not express S100 proteins significantly in the pluripotent state. Stem cell markers such as OCT4, NANOG, SOX2 are more elevated at this stage (12). Early neuron markers begin to increase in the first 3–5 days after RA is applied to the cells. S100B expression begins from day 7–14. In a study in the literature, S100B expression was used as an indicator of glial differentiation while examining the neuronal and glial orientation of embriyonic stem cells such as NT2 and NCCIT with RA (13).

NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) is a transcription factor family that plays a role in many critical processes of the cell such as stress response, immune response, inflammation, apoptosis, cancer and neurodegenerative diseases (14). Following RA treatment, the expression of the NF-κB protein reduced. Because during development, this protein inhibits anti-apoptotic signaling and cell proliferation.

Demonstrating changes in cell morphology and expression of S100B and NF-κB proteins after administration of retinoic acid to testicular embryonal carcinoma cells is an important step toward a deeper understanding of how these proteins play a role in cancer cells and how they contribute to cancer molecular biology.

MATERIALS AND METHODS:

Cell Culture:

The human testicular embryonal cancer cell line CRL-2073 (NCCIT, Abcam) was used in this study. They were cultivated in 6 well plates in RPMI-1640 medium (L-glutamine, HEPES, Gibco) that contained 5% fetal bovine serum (FBS, Capricorn) and 1% antibiotic (penicillin-streptomycin, Gibco). 37°C and 5% CO_2 conditions were used to incubate the cells.

Retinoic Asid (RA) Induced Differentiation:

After the cells are grown in a suitable culture medium, RA was applied at the dose determined according to the literature to study groups (15,16). The cells were exposed to 10 μM RA for 10 days in order to demonstrate whether the cells had differentiated into nerve cells. Every day following the application, the vitality of the cells was assessed. To control groups no application was performed.

Crystal Violet Staning:

Stanining CRL-2073 cells with crystal violet after RA administration is a very meaningful method to evaluate the response of the cells in terms of survival, adhesion and differentiation. Coverslips were placed in 12 well plates and cells were cultured. 4 % paraformaldehyde (PFA) was used to fix the cells and wait for 10 minutes. Then the cells were washed with PBS and the round coverslips were placed on the slide. Cells were passed through 100%-96%-75% alcohol series and then washed with distilled water. Slides were incubated in working solution (Crystal violet-mos lab) for 1 hour. Then the slides were cleaned under tap water for 5 minutes. They were passed through 75%-96%-100% alcohol series for dehydration. In the last step, they were kept in xylene for 30 minutes and covered with entellan. Photographs were taken using a light microscope (Nikon). The purpose of performing this staining is to observe whether the cells survive after RA application, their adhesion to the surface and whether RA has a toxic effect.

Luxol Fast Blue Staninig:

Luxol Fast Blue (LFB) is a histological dye that is specifically used to stain myelin sheaths and nerve tissue. It usually gives myelin sheaths a blue colour, which makes it easier to examine neuronal structures under a microscope. This makes it feasible to investigate the location and composition of the myelin sheath within the nervous system. Action potentials can be swiftly transmitted by nerve cells because to the insulating layer called myelin. Degeneration or injury to myelin sheaths can also be demonstrated by LFB staining. After the cells received the recommended dosage of retinoic acid, LFB labeling was used in our investigation to check for cell differentiation. Myelin/myelinated axons and Nissl granules are intended to be demonstrated via LFB staining (Bio Optica, 24192). On coverslips set inside 12-well plates, cells were sown. % 4 paraformaldehyde was used to fix the cells. Ten drops of the kit's reagent A were put to the slide in a humid setting, and the slide was then incubated for the entire night at 56°C. To get rid of the crystal remnants of reagent A, the preparation was rinsed with 95% ethanol the following day and then distilled water. After adding ten drops of reagent B to the mixture, 30 seconds were spent waiting.

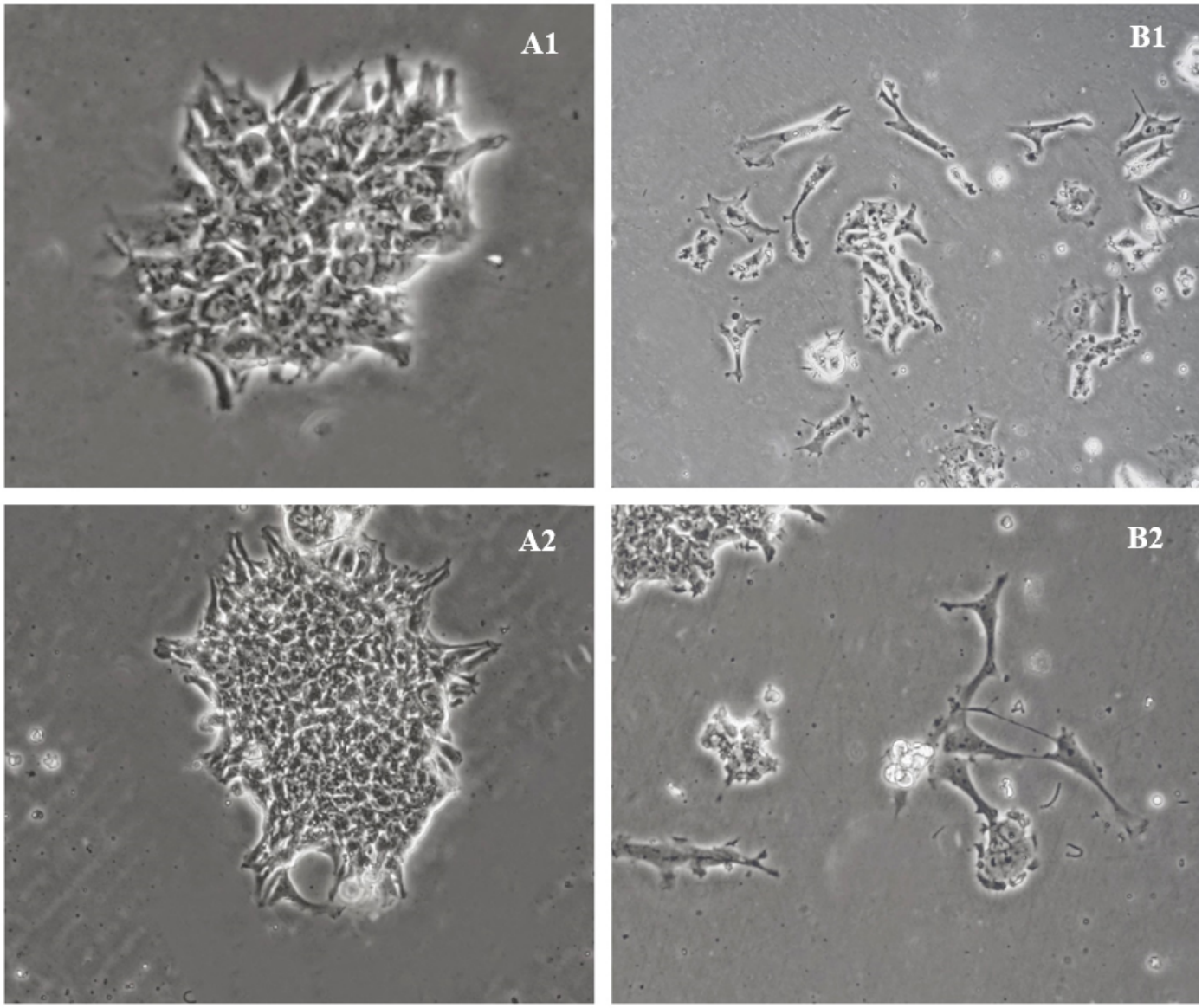

Figure 1. Morphological images of CRL-2073 cells before and after RA application. (A1 and A2: CRL-2073 cells that were not exposed to RA. B1: CRL-2073 cells' after 7 days of 10 μM RA treatment and B2: CRL-2073 cells' after 10 days of 10 μM RA treatment Magnification: 200X).

Myelin fibers were rinsed with distilled water after being distinguished with 70% ethanol until they turned blue. Ten drops of reagent C and five drops of reagent D were applied to the slide once the humid environment was restored. For 20 minutes, the incubation box was kept at 56°C. Nissl granules were dyed pale pink after being distinguished in 95% ethanol. Following ethanol dehydration, they were stored in xylene before being covered with entellan. A light microscope was used to take pictures (Nikon).

Immunocytochemistry:

Immunocytochemistry staining was performed to demonstrate the localization and expression of S100B and NF-κB proteins in the nucleus and cytoplasm before and after RA application in the human testicular embryonal carcinoma cell line (CRL-2073). In 12-well cell plates, cells were cultured on circular coverslips for immunocytochemistry staining. The cell medium was removed and PBS was used to wash the cells when they had attained a specific density (about %80-90). After that, the cells were fixed for 15 minutes using 4% PFA. After drawing with a pappen, the coverslips that were put on the slide were cleaned with PBS. To avoid nonspecific binding, they were incubated with blocking solution for one hour. They were given another PBS wash following incubation. The coverslips were covered with primary antibodies S100B (St John's, 1:50) and NF-κB (Santa Cruz, 1:50), and incubated for the entire night at +4°C. Primary antibodies were removed and cleaned with PBS the following day. After that, they spent an hour being treated with a biotinylated secondary antibody. They were incubated with Streptavidin solution for half an hour after being cleaned with PBS. AEC solution was added on them in order to make the reaction visible, and the incubation period was changed by observing under a microscope. Following washing, they were incubated for 30 seconds with Mayer's Hematoxylin to stain the nucleus and coated with a water-based covering medium. Assessments were conducted using a light microscope.

RESULTS:

Morphological effects of RA application:

CRL-2073 cells were treated with RA (10 μM) to induce differentiation until day 10. At RA treatment, the growth of

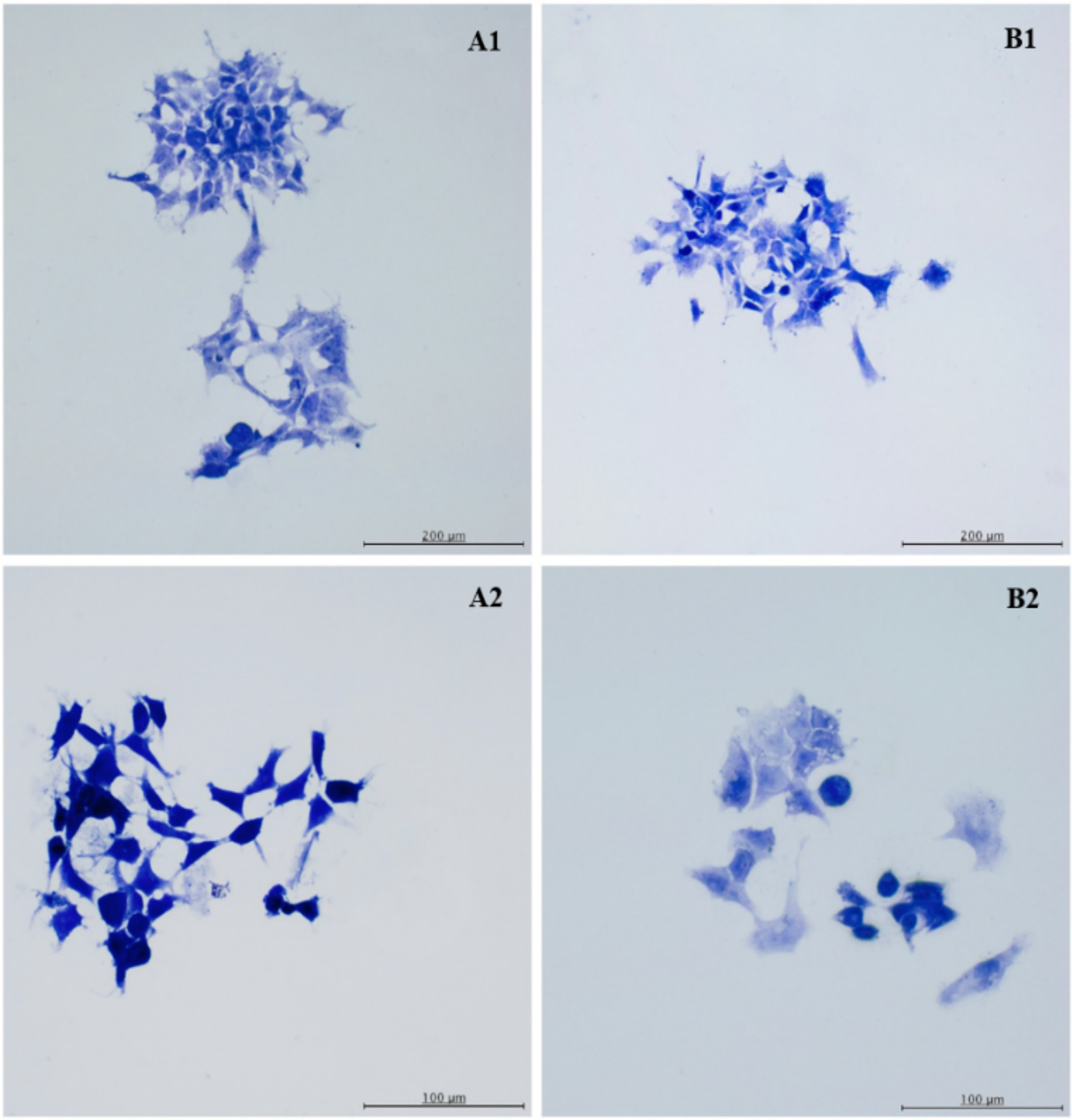

Figure 2. Crystal violet staining images of CRL-2073 cells before and after RA application. (A1 and A2: CRL-2073 cells that were not exposed to RA. B1: CRL-2073 cells' after 7 days of 10 µM RA treatment and B2: CRL-2073 cells' after 10 days of 10 µM RA treatment)

these cells slows in response and differentiation follows. As CRL-2073 cells differentiate, their spherical shape is significantly changed during differantiation (Figure 1). Some of the flattened cells had branched, extended cytoplasmic processes, which are characteristic of the shape of neurons (5,16).

Crystal Violet Stanining results:

When the staining results of the control group (not exposed to RA) and the RA group were compared, the number of stained cells was examined to understand whether RA had a toxic effect. And as a result, it was observed that the cell number in the RA group decreased compared to the control group (Figure 2). In the RA group, the staining intensity generally decreased. Because the cells differentiate, cell proliferation rate slowed down and some of them entered to the apoptotic process.

Luxol Fast Blue Staining results:

Human testicular embryonal carcinoma cells are not stained well with Luxol fast blue because this dye specifically targets lipid structures in myelinated nerve tissue. Human testicular embryonal carcinoma cells are pluripotent cells that do not contain myelin. Therefore, staining is much less

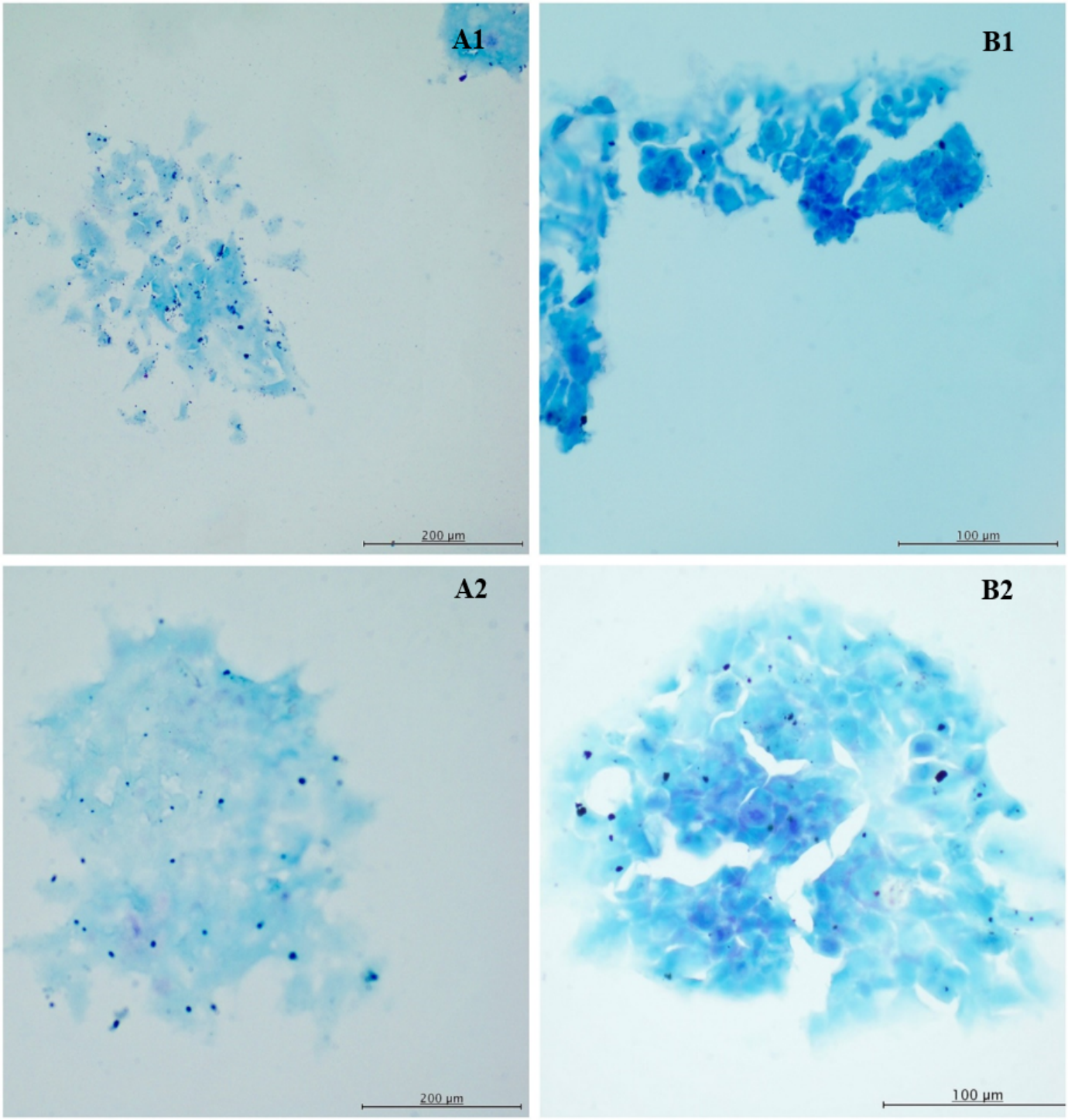

Figure 3. Luxol Fast staining images of CRL-2073 cells before and after RA application. (A1 and A2: CRL-2073 cells that were not exposed to RA. B1: CRL-2073 cells' after 7 days of 10 µM RA treatment and B2: CRL-2073 cells' after 10 days of 10 µM RA treatment).

in the group that was not given retinoic acid than in the group that was given retinoic acid (Figure 3). The luxol fast blue staining results showed that CRL-2073 cells started to morphologically change into nerve cells after RA.

Immunocytochemistry results:

After RA application, S100B and NF-κB protein expression has not been examined in human testicular embriyonal carcinoma cell lines before. S100B was expressed at higher rates in RA-treated cells compared to untreated cells. NF-κB was expressed at a lower rate in RA-applied cells compared to those not applied (Figure 4).

DISCUSSION

This Testicular embryonal carcinoma is a malignant tumor that develops in the testes and usually originates from germ cells. The biological features, intracellular signaling

pathways and protein expressions of these tumors are important for diagnosis and treatment strategies.

An essential substance called retinoic acid (RA) causes differentiation and controls the expression of several genes in testicular embryonal carcinoma cells, particularly in the NT2/D1 cell line. Retinoic acid receptors (RAR) and retinoid X receptors (RXR) are often the mechanisms by which RA

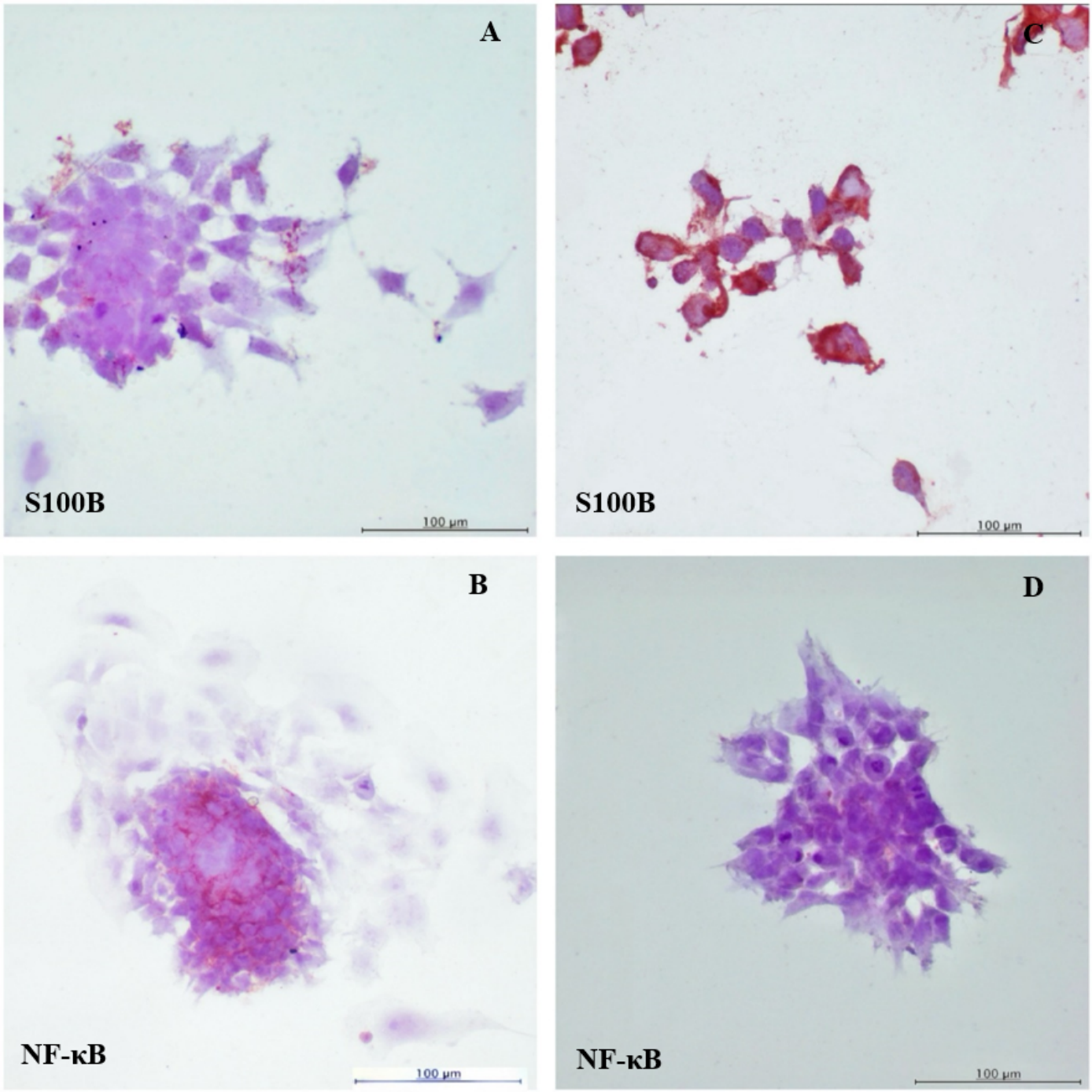

Figure 4. Immunocytochemical demonstration of S100 and NF-κB protein in CRL-2073 cells. (A: S100B staining of CRL-2073 cells that were not exposed to RA. B: S100B staning of CRL-2073 cells' after 10 days of 10 µM RA treatment. C: NF-κB staining of CRL-2073 cells that were not exposed to RA. D: NF-κB staining of CRL-2073 cells' after 10 days of 10 µM RA treatment).

works (6). RA has the ability to differentiate the CRL-2073 cell line, a pluripotent cell model derived from testicular embryonal cancer. Retinoic acid can cause embriyonic stem cells to differentiate. When CRL-2073 cells are treated with RA, they lose their pluripotency, change morphologically, flattening and sometimes acquiring the branched and elongated cytoplasmic processes characteristic of neurons, and show structural alterations of glycosaminoglycan chains made by the heparin/heparin sulfate and chondroitin sulfate/dermatan sulfate pathways. Furthermore, RA significantly suppresses carcinogenesis and clonal development in stem cells generated from cancer (17). Thus, RA-mediated CRL-2073 cell differentiation provides a valuable paradigm for research on cancer and pluripotency of stem cells.

In cryistal violet staining, high staining of retinoic acid treated group indicates that the cells were viable and attached to the surface. This indicates that RA is well tolerated by the cells. Low staining indicates that the RA dose may be toxic due to cell death or loss of adhesion. Crystal violet is a good stain for observing the effect of the differentiation process on survival because it shows only those that are attached to the surface. In our study, as a result of staining with cristal violet, we observed that the

number of cells in the RA group decreased compared to the control group and the staining intensity generally decreased.

Luxol fast blue dye is an aniline-based dye. It binds to the phospholipids in the myelin sheath and stains the structures blue. Since neuronal bodies do not contain myelin, they hold little dye and appear pale. This dye is especially used in studies on differentiation, neuronal development or myelin degeneration. When RA is given to embryonal carcinoma cells, if neuronal differentiation is not at a very advanced level, Luxol fast blue may not be expressed very high.

In our study, as result of staining with luxol fast blue, it is shown that CRL-2073 cells began to morphologically change into the nerve cells.

S100B and NF-κB proteins are two important molecules that regulate different biological processes in such tumors. Some studies suggest that the NF-κB transcription factor may regulate the expression of S100 proteins, such as S100A6 (18). These findings suggest that NF-κB may affect the expression of S100 proteins and that this mechanism may also apply to testicular embryonal carcinoma cells. However, further research is needed to directly demonstrate this relationship in testicular embryonal carcinoma cells. Expression of S100B and NF-κB proteins in human testicular embryonal carcinoma cells is directly related to biological processes such as cell pluripotent status, differentiation orientation and proliferation. The potential interaction between these two proteins may affect the biological behavior of testicular embryonal carcinoma cells. S100B protein expression increased after RA application. Because this protein functions as a glial cell differentiation marker and its expression increases in neuronal differentiation states. NF-κB protein expression decreased after RA application. Because this protein suppresses cell proliferation and anti-apoptotic signaling during differentiation.

NT2/D1 cells are a well-studied model of human testicular embryonal carcinoma cells. It has been shown that after RA is applied to these cells, pluripotency factors are reduced, proliferation is suppressed, and NF-κB activity is reduced. In other words, the decrease in NF-κB activity as the cells differentiate is associated with the loss of their proliferative character. RA treatment caused a decrease in cyclin D1 protein levels and a G1 phase in NT2/D1 cells. This indicates that cell proliferation is suppressed and the differentiation process has begun (19,20). In later stages of RA administration, nuclear localization of NF-κB (especially the p65 subunit) may decrease (21). This means that NF-κB is less transcriptionally active. The differentiation process initiated by RA proceeds together with the suppression of the NF-κB pathway, causing cells to lose their proliferative and pluripotent identity.

As a result of retinoic administration to embryonal carcinoma cells, the expression of pluripotency genes such as OCT4, NANOG, and SOX2 is reduced (22). This decrease indicates that the cells are no longer pluripotent and have entered the differentiation process. With this change, proliferation slows down because pluripotent cells typically divide rapidly. Publications in the literature are generally about differentiation genes such as OCT4, NANOG, and SOX2 and their expressions. In this study, we aimed to observe how histologically RA application affects cell morphology before the genetic level and how RA application affects S100B and NF-κB protein levels.

When we look at the literature on the differentiation studies of CRL-2073 cells with retinoic acid, it has been shown that the cellular proteoglycan composition of these cells is involved in the differentiation mechanism in response to retinoic acid. Retinoic acid is crucial for the early embryo's pre-patterning. By inducing differentiation in vitro, retinoic acid can make pluripotent and multipotent cells more lineage-restricted (15).

Redirecting cell behavior is necessary for new medical therapy approaches, such as inducing cancer cell differentiation to prevent metastasis. Embryonic carcinoma cells make up teratocarcinomas, which are malignant germ cell tumors. They are the cancerous counterparts of healthy pluripotent embryonic cells from the embryo's preimplantation phase. More than two dozen well-differentiated adult tissues from all three germ layers, including the brain, muscle, bone, teeth, bone marrow, eyes, secretory glands, skin, and intestine, as well as placental and yolk sac tissue, are mixed together in tumors formed by single embryonal carcinoma cells from teratocarcinomas. The use of in vitro grown embryonal carcinoma cells as a model system for the study of early embryonic development has been encouraged by their capacity to produce ordered structures that resemble the growing embryo. It has been demonstrated that the extensively researched CRL-2073 cell line, which was generated from a mediastinal mixed germ cell tumor, may develop into extraembryonic cell lineages and derivatives of the three embryonic germ layers (ectoderm, mesoderm, and endoderm). The CRL-2073 responds to retinoic acid, which causes cellular differentiation and the elimination of pluripotent oligosaccharide surface antigens. These factors, along with their ease of manipulation, make CRL-2073 cells a valuable model to measure the concurrent alterations in the glycan profile following RA treatment. This allows for the identification of promotive and/or restrictive changes linked to the morphogen's action of inducing loss of pluripotency and increased lineage restriction. CRL-2073 cells can develop into representatives of every germ layer, much like pluripotent embryonic stem cells can. As a reflection of its function in the early development of tissue, RA therapy guides these cells along certain pathways. As a result, the complex circuitry controlling cell fate with environmental effects for pluripotent and differentiated cell states will be clarified by these analyses together with transcriptome, epigenome and proteome profiles. These differentiation mechanisms are of great importance for pluripotent cells such as CRL-2073 (15).

Although there is no study directly examining NF-κB expression in CRL-2073 cells, the existence of NF-κB activity in general testicular tissue and the role of NF-κB in the differentiation processes of embryonal carcinoma cells indicate that further research is needed on this subject. Our study is important because it is fundamental study in this field.

Future studies in this area will help us better understand how NF-κB plays a role in embryonal carcinoma cells and how this knowledge can be integrated into clinical practice.

Measuring S100B and NF-κB levels in human testicular embryonal carcinoma cells treated with RA may be of great importance in understanding cellular differentiation, proliferation, apoptosis, and inflammation. These two factors have distinct but interactive biological roles, and understanding how these pathways are regulated by RA administration is particularly valuable in terms of tumor biology and differentiation mechanisms. Examining these two factors together allows for a more comprehensive

understanding of the effects of RA treatment on both differentiation (S100B) and proliferation/apoptosis control (NF-κB) in tumor cells.

CONCLUSION

In conclusion at this study we demonstrated the morphological changes and S100B and NF-κB expressions of testicular embryonal carcinoma cells after RA stimulation. This is a pioneering study in this field and we believe it will shed light on the field of cancer biology.

Ethics Committee Approval: Ethics committee approval was not required for this study because of there was no study on animals or humans. Commercial cell lines were used in this study and therefore ethical approval was not required.

Informed Consent: Informed consent was not required for this study because of there was no study on animals or humans.

Authorship Contributions: Idea/Concept: ŞK and HN, Design: ŞK, Supervision: HN, Data Collection and Processing: ŞK Analysis or Interpretation: ŞK and H, Literature Search: ŞK, Writing: ŞK and HN,Critical Review: ŞK and HN, References and Fundings:-Materials: -.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare that they have no relevant financial.

REFERENCES

1. Reuter V. Origins and molecular biology of testicular germ cell tumors. Modern Pathology 2005; 18(S2), S51–S60.
2. Baird D, Meyers G, Hu J. Testicular cancer: Diagnosis and treatment. American Family Physician 2018; 97(4), 261-8.
3. Yamanaka S. Pluripotent stem cell-Based cell therapy-Promise and Challenges. Cell Stem Cell 2020; 523-31.
4. Teshima S, Shimosato Y, Hirohashi S et al. Four new human germ cell lines. Lab Investig 1988; 59:328–36.
5. Damjanov I, Horvat B, Gibas Z. Retinoic acid-induced differentiation of the developmentally pluripotent human germ cell tumor-derived cell line, NCCIT. Lab Investig 1993; 68:220–32.
6. Soltanian S, Sheikhbahaei M, Ziasistani M. Phytol down regulates expression of some cancer stem cell markers and decreases side population proportion in human embryonic carcinoma NCCIT cells. Nutrion and Cancer 2021; 1520-33.
7. Janesick A, Cherie W, Blumberg B. Retinoic acid signaling and neuronal differentiation. Cell Mol Life 2015; 72 (8): 1559-76.
8. Chen H, Xu C, Jin Q et al. S100 protein family in human cancer. American Journal of Cancer Research 2014, 4(2), 89-115.
9. Gonzalez L, Garrie K, Turner M. Role of S100 proteins in health and disease. Biochmica et Biophysica Acta (BBA)-Molecular Cell Research 2020;118677.
10. Tsutsui Y, Nogami T, Kashiwai A et al. Induction of S100b (beta beta) protein in human teratocarcinoma cells. Cell Differ 1987; 21(2):137-45.
11. Wang S, Rosegren L, Franlund M et al. Bcl-2 expression regulates cell sensivity to S100beta-mediated apoptosis. Brain Res Mol Brain Res 1999; 70(1);167-76.
12. Sung B, Do H, Park S et al. Regulation of OCT4 gene expression by liver receptor homolog-1 in human embryonic carcinoma cells. Biochemical and Biophysical Research Communications 2012; 427(2), 315–20.
13. Coyle D, Li J, Baccei M. Regional differentiation of retinoic acid-induced human pluripotent embryonic carcinoma stem cell neurons. Plos One 2011; 20(6);16174.
14. Imran M, Park J, Lim I. Stress-induced NF-KB activation differentiates promyelocytic leukemia cells to macrophages in response to all-trans-retinoic acid. Cell Signaling 2015; 27(3):694-706.
15. Gasimli L, Stansfield H, Nairn A et al. Structural remodeling of proteoglycans upon retinoic acid-induced differentiation of NCCIT cells. Glycoconj 2013; 30(5):497-510.
16. Park S, Do H, Ha W et al. Differential Expression of ETS Family Transcription Factors in NCCIT Human Embryonic Carcinoma Cells upon Retinoic Acid-Induced Differentiation. Biological and Pharmaceutical Bulletin 2014; 37(4), 659–65.
17. Niu C, Li M, Ni Y et al. Effect of all-trans retinoci acid on the proliferation and differentiation of brain tumor stem cells. Journal of Experimental &Clinic Cancer Reserach 2010; 29(1):113.
18. Joo J, Kim J, Lee Y et al. Involment of NK-kappaB in the regulation of S100A6 gene expression in human hepatoblastoma cell line HepG2. Biochem Biophys Res. Commun 2003; 307(2): 274-80.
19. Spinella M, Freemantle S, Sekula D et al. Retinoic acid promotes ubiquitination and proteolysis of cyclin D1 during induced tumor cell differentiatşon. Cell Biology Metabolism 1999;22013-8.
20. Baldassarre G, Bruni A, Sandemenico C et al. Retinoic acid induces neuronal differentiation of embryonal carcinoma cells by reducing proteasome dependent proteolysis of the cyclin dependent inhibitör p27. Cell Growth Differ 2000; 11(10): 517-26.
21. JH S, Bours N, JA M et al. Retinoic acid induction of majör histocompatibility complex class I genes in NTera-2 embriyonal carcinoma cell involves induction of NF-kappa B (p50-p6 5) and retinoic acid receptor beta retinoid X receptor beta heterodimers. Molecular and Cellular Biology 1993; 13(10): 6157-69.
22. Park S, Do H, Ha W et al. Transcriptional regulation of OCT4 by the ETS transcription factor ESE-1 in NCCIT human embriyonic carcinoma cells. Biochemical and Biophysical Reserach Communications 2014; 984-90.