onco

MDPI

Article

The Different Effects of Noradrenaline on Rhabdomyosarcoma and Ewing's Sarcoma Cancer Hallmarks—Implications for Exercise Oncology

Peter Weeber [1,†], Stephanie Bremer [1,†], Jonas Haferanke [2], Carla Regina [3], Martin Schönfelder [1], Henning Wackerhage [1,*,‡] and Irene von Luettichau [3,‡]

[1] Professorship of Exercise Biology, TUM School of Medicine and Health, Technical University of Munich, 80809 Munich, Germany; peter.weeber@tum.de (P.W.); stephanie.bremer@tum.de (S.B.); martin.schoenfelder@tum.de (M.S.)
[2] Institute of Preventive Pediatrics, TUM School of Medicine and Health, Technical University of Munich, 80992 Munich, Germany; jonas.haferanke@tum.de
[3] Kinderklinik München Schwabing, Department of Pediatrics and Children's Cancer Research Center, TUM School of Medicine, Technical University of Munich, 80804 Munich, Germany; carla.regina@tum.de (C.R.); irene.teichert-vonluettichau@mri.tum.de (I.v.L.)
* Correspondence: henning.wackerhage@tum.de
† These authors contributed equally to this work.
‡ These authors also contributed equally to this work.

Simple Summary: High-impact publications have reported both the beneficial and harmful effects of catecholamines on cancer hallmarks and outcomes—a contradiction that is underappreciated and poorly explained. Here, we aimed to investigate whether differences in adrenergic receptor isoform expression can explain different cancer hallmark responses to catecholamines, since cancer cells can vary greatly in the expressions of nine adrenergic receptors. For this purpose, we cultured two cancer cell lines that systematically differ in their adrenergic receptor expressions: A673 cells, which express α1D-, α2C-, β1-, and β3-adrenergic receptors, and RD sarcoma cells, which barely express any adrenergic receptors. The cells were treated with noradrenaline to elucidate the effects of noradrenaline exposure on cell proliferation, migration, and cAMP signaling. While the A673 cells responded to noradrenaline treatment with decreased cell numbers, cell proliferation and migration, and increased cAMP signaling, the RD cells did not respond to noradrenaline. Therefore, our findings indicate that the adrenergic receptor isoform expressions might indeed explain why cancers can respond differently to increases in catecholamine concentrations due to, e.g., a bout of exercise, psychosocial stress, surgery, or drugs such as β-blockers.

Abstract: Background: Exercise has beneficial effects on cancer and its treatment, but the underlying mechanisms are poorly understood. Some studies have linked the positive impact of exercise to catecholamine signaling. In contrast, cancer stress studies have typically reported that catecholamines worsen cancer hallmarks and outcomes. Here, we aimed to investigate whether adrenergic receptor isoform expression can explain the contradictory effects of catecholamines in cancer. Methods: We cultured two pediatric sarcoma cancer cell lines that either express (A673 cell line) or do not express (RD cell line) adrenergic receptors. The cells were treated with a 5× dilution series of noradrenaline to assess the effects of noradrenaline on cell numbers. After these dose-finding experiments, we treated both cancer cell lines with 60 μM noradrenaline to examine its effect on cell proliferation and migration and cAMP signaling. Results: Treatment with 60 μM noradrenaline significantly decreased the cell numbers by 61.89% ± 10.36 ($p \leq 0.001$), decreased cell proliferation by 15.88% ± 6.76 ($p \leq 0.05$), decreased cell migration after 24 h ($p \leq 0.001$), and increased cAMP concentrations 38-fold ($p \leq 0.001$) in the A673 cells, which express adrenergic receptors, but not in the RD cells, which do not express adrenergic receptors. Conclusions: Our results indicate, as a proof of principle, that the effects of catecholamines on cancer progression and metastasis might depend on the expressions of the nine adrenergic receptor isoforms. As cancers express adrenergic and other receptors differentially, this

check for updates

Citation: Weeber, P.; Bremer, S.; Haferanke, J.; Regina, C.; Schönfelder, M.; Wackerhage, H.; von Luettichau, I. The Different Effects of Noradrenaline on Rhabdomyosarcoma and Ewing's Sarcoma Cancer Hallmarks—Implications for Exercise Oncology. *Onco* **2024**, *4*, 397–411. https://doi.org/10.3390/onco4040028

Academic Editor: Constantin N. Baxevanis

Received: 27 September 2024
Revised: 26 October 2024
Accepted: 29 October 2024
Published: 7 November 2024

has implications for the response of cancers to exercise, stress, and medication and may help to further personalize cancer treatments.

Keywords: cancer; tumor cells; rhabdomyosarcoma; Ewing's sarcoma; catecholamines; noradrenaline; adrenergic receptors

1. Introduction

Physical activity reduces the risk of developing cancer [1], delays disease progression [2], improves survival [3], supports the management of treatment side effects [4], and increases the overall fitness and well-being of cancer patients. Given this high potential of physical activity in improving both disease-specific outcomes and treatment tolerability, exercise has been proposed as an adjunct therapy for cancer patients [5], and it is recommended by several cancer associations, such as the American Cancer Society [6] and the American College of Sports Medicine [7]. Nevertheless, the mechanisms by which exercise affects cancer are still incompletely understood. What we do know, however, is that exercise changes the blood concentrations of myokines [8], metabolites [9], immune cells [10], and hormones [11] and that these factors can potentially affect cancer hallmarks if cancer cells express receptors or targets for them.

The two stress hormones adrenaline (epinephrine) and noradrenaline (norepinephrine) are synthesized and secreted by the medulla of the adrenal gland [12]. During exercise, catecholamine concentrations can increase up to >20 fold depending on the duration and intensity of exercise [12], which makes adrenaline and noradrenaline the two main hormones whose concentrations increase substantially during physical exertion. For instance, adrenaline concentrations have been shown to increase from 0.6 nmol/L at rest to 4 nmol/L after 10 intervals of 6 s sprints [13]. Similarly, noradrenaline levels have been found to increase from 1.7 nmol/L at rest to 25 nmol/L after exercise [13]. These exercise-induced increases in catecholamine concentrations can promote the accumulation of natural killer cells [14], an increase in antitumor immunity, more CD8+ T cell tumor infiltration [15], and the activation of the Hippo pathway [16], thereby suppressing tumor growth.

However, exercise is only one of the multiple stimuli that can affect adrenaline and noradrenaline concentrations. Psychosocial stress (i.e., public performance), physiological stress (i.e., pain), and environmental stress (i.e., cold exposure) have also been reported to alter catecholamine concentrations [17]. Additionally, drug and substance usage is known to modulate catecholamine signaling by targeting adrenergic receptor isoforms [18]. Surprisingly, while exercise, which is associated with temporary increases in catecholamines, suppresses tumor growth [14,19], chronic stress-induced catecholamine concentration increases appear to worsen cancer hallmarks and outcomes [20,21]. The drug administration of, i.e., β-blockers, is mostly associated with beneficial effects, that are, however, restricted to specific cancer types [22,23]. We term the contradiction of catecholamine signaling having both beneficial and adverse effects on cancer as the "cancer catecholamine conundrum" [12].

We hypothesize that the variable and often opposite effects of catecholamine signaling on cancer hallmarks and progression are mediated by the variable adrenergic receptor isoform expression on cancer cells [12]. Adrenergic receptors are a class of G protein-coupled receptors (GPCRs) that bind to the catecholamines adrenaline and noradrenaline. The receptors are classified into three main adrenergic receptor families: alpha-1 (α-1), alpha-2 (α-2), and beta (β), with three receptor isoforms each [24]. Depending on the G protein complex that is coupled to the receptor, catecholamine binding can initiate different and even opposite cellular responses to noradrenaline and adrenaline receptor binding [25]. We propose that cancer cells with distinct adrenergic receptor isoform expression profiles may respond differently to catecholamine exposure. Specifically, we expect that cancer cells expressing higher levels of adrenergic receptors will exhibit altered proliferation, migration,

and signaling responses upon noradrenaline treatment, whereas cancer cells with low receptor expression may not respond to noradrenaline. This variability in adrenergic receptor expression could provide a mechanistic explanation for the contradictory effects of catecholamines on cancer hallmarks, as seen in exercise- and stress-related cancer studies.

The aim of the present study, therefore, was to investigate whether differences in adrenergic receptor isoform expressions can explain different cancer hallmark responses to catecholamines. For this purpose, we compared the A673 Ewing's sarcoma cancer cell line, which generally expresses high amounts of α1-, α2-, and β-adrenergic receptors, with the RD rhabdomyosarcoma cancer cell line, which expresses low levels of adrenergic receptors. In doing so, we aimed to address the following research question: Do the cell proliferation and migration and cAMP signaling responses to noradrenaline treatment differ between A673 and RD sarcoma cells?

2. Methods

2.1. Cell Lines and Culturing

We selected the A673 Ewing sarcoma and the RD rhabdomyosarcoma cancer cell lines based on their adrenergic receptor expressions in the cancer cell line encyclopedia (CCLE; [26]). Both cell lines belong to the group of pediatric sarcomas but differ in their adrenergic receptor isoform expressions. While A673 cells express α1D-, α2C-, β1-, and β3-adrenergic receptors, RD cells do not express α1-, α2-, or β-adrenergic receptors [27].

The A673 cell line was obtained from ACTT. The RD cell line was obtained from Dr. Henning Wackerhage. We cultured the A673 and RD cell lines (DSMZ, Braunschweig, Germany) in RPMI 1640 (A673 cells) and DMEM/F12 medium (RD cells) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin (all obtained from Life Technologies, Grand Island, NY, USA). The cells were authenticated (Eurofins Genomics Europe Applied Genomics GmbH, Ebersberg, Germany) and frequently tested for mycoplasma contamination. We sub-cultured the cells twice every week at 80% confluence and used the cells for experiments up to passage 30.

For the treatment of the cells, we prepared a noradrenaline stock solution of 3 mM by dissolving 12.3 mg noradrenaline (noradrenaline-hydrochloride in crystalline form was obtained from Merck, Darmstadt, Germany) in 20 mL of DEPC-treated H_2O (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA). We stored 1 mL aliquots of the stock solution at −20 °C for a maximum of three months until they were used for the experiments. We diluted the noradrenaline stock solution to the desired concentration with growth medium. The cells treated with growth medium only, as a control, were treated with growth medium that contained DEPC-treated H_2O in the same ratio as the noradrenaline solution.

2.2. RNA Sequencing and Transcriptome Analysis

We analyzed the transcriptomes of the A673 and RD sarcoma cells to (1) confirm the CCLE adrenergic receptor expression data and (2) analyze the effects of noradrenaline on gene expressions in the A673 and RD cell lines. For this purpose, we seeded 2×10^6 cells per Petri dish (150 mm) in quadruplicate and incubated the dishes at 37 °C and 5% CO_2 for 24 h. After the cells were adhered to the plastic, we treated them with 60 µM of noradrenaline for 24 or 48 h or with growth medium for 48 h (control). After washing with phosphate-buffered saline (PBS), we pelleted the cells with a cell scraper. The total RNA was extracted using Trizol reagent (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. Transcriptome sequencing was conducted by Novogene (Novogene UK Company Limited, Cambridge, UK). We calculated the FPKM (fragments per kilobase of transcripts per million fragments mapped) value of each gene to normalize the read count based on the gene length and the total number of mapped reads.

2.3. Sulforhodamine B Assay

To measure the effects of noradrenaline on the A673 and RD sarcoma cell numbers, we treated the cells for 24, 48, and 72 h with different concentrations of noradrenaline or growth medium only (control) and quantified the cell numbers using the sulforhodamine B (SRB) assay. This assay is commonly used in cancer cell lines to estimate cell numbers and to test for drug toxicity [28]. For this purpose, we seeded A673 and RD cells in triplicates in three 24-well plates each, with 50,000 cells per well. After 24 h, when the cells were adhered to the plastic, we tested different concentrations of noradrenaline in a 5× dilution series: 0.3 mM, 60 µM, 12 µM, 2.4 µM, 0.48 µM, and 96 nM. We fixed the cells after 24, 48, and 72 h to the tissue-culture plates with 10% trichloroacetic acid and incubated the plates for at least one hour at 4 °C. After dyeing the cells with 0.05% SRB, washing with 1% acetic acid, and air-drying, we added 10 mM Tris base solution to each well to solubilize the protein-bond dye. Photometric measurements were conducted using the Tecan Infinite™ M200 plate-reader (Tecan Trading AG, Männedorf, Switzerland) at 530 nm. The experiment was repeated three times, and we present the results of one run representing all runs.

2.4. Proliferation Assay

To measure the effects of noradrenaline on the cell proliferation of A673 and RD sarcoma cells, we treated the cells with 60 µM of noradrenaline for 72 h. The noradrenaline concentration and treatment duration in this proliferation assay were selected based on the results of the preliminary SRB assay. After seeding 20,000 cells per well in duplicate in a 24-well plate, we incubated the cells for 24 h until they adhered to the plastic. Cell proliferation was assessed by 5′-ethynyl-2′-deoxyuridine (EdU) incorporation using the EdU Cell Proliferation Kit for Imaging (EdU-Click 555, baseclick GmbH, Neuried, Germany) according to the manufacturer's protocol. We added the EdU solution to the medium during the last 4 h of the 72 h incubation period. Further, we dyed cells with bisBenzimide H 33342 trihydrochloride (B2261, Sigma-Aldrich, St. Louis, MO, USA) to visualize the living cells and took fluorescence microscopy images with the Zeiss Axiovert 100 microscope (Carl Zeiss AG, Oberkochen, Germany) at 10× magnification. In each image, we defined three random view fields and counted at least 2000 cells per experiment.

2.5. Migration Assay

We assessed the effects of noradrenaline on the cell migration of A673 and RD sarcoma cells using 35 mm Culture-Insert 2 Wells in µ-Dishes (ibidi GmbH, Gräfelfing, Germany). As stated above, the noradrenaline concentration and treatment duration were selected based on the results of the preliminary SRB assay. First, we seeded cells at a density of 7×10^5 per mL (70 µL volume) in each of the two chambers of the silicone insert and incubated the dishes at 37 °C and 5% CO_2 for 24 h. The cells were incubated in growth medium without FBS in order to starve the cells and to induce a reduced proliferative state. After the cells adhered to the plastic and reached up to 100% confluence, we removed the silicone insert to uncover the 500 µm cell-free gap. We then treated the cells with 60 µM of noradrenaline or growth medium (control) and took microscopy images with the Zeiss Axiovert 100 microscope (Carl Zeiss AG, Oberkochen, Germany) at 10× magnification immediately after treatment as a baseline. In the next step, we took microscopy images after 24, 48, and 72 h of treatment in each dish and analyzed the images by the percentage of scratch open area using the web-based quantitative image analysis software FastTrack AI software number 1.1 (ibidi GmbH, Gräfelfing, Germany). The cells were seeded on three different dates with three technical replicates each. We present the results of one run representing all runs.

2.6. cAMP Signaling Assay

To analyze the effects of noradrenaline on the cyclic adenosine monophosphate (cAMP) signaling pathway of A673 and RD sarcoma cells, we assessed the cellular concentrations of cAMP after treating the cells with 60 µM or 20 nM of noradrenaline or growth medium

only (control). We quantified the cAMP concentrations using a cAMP competitive enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA). For this purpose, we seeded 3.5×10^5 cells per well in 24-well plates (one plate for each cell line) and incubated the cells at 37 °C and 5% CO_2 for 24 h. After the cells were adhered to the plastic, we treated the cells with 60 µM or 20 nM of noradrenaline or with growth medium for 5, 15, and 30 min. Cell lysis was then induced by 0.1 M hydrogen chloride (HCl) and 1% Triton X-100. After ten minutes of incubation at room temperature, we centrifuged the plates at 600× *g* at room temperature for another ten minutes and used the supernatant duplicates in the assay according to the manufacturer's protocol. The optical density values were analyzed using a Tecan Infinite™ M200 plate-reader (Tecan Trading AG, Männedorf, Switzerland) at 405 nm with correction at 580 nm. We calculated the cAMP concentrations by data interpolation based on the standard curve's exponential function in Microsoft Excel™ Office 2016 (Microsoft, Redmond, WA, USA).

2.7. Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9.2.0 (GraphPad, San Diago, CA, USA). The data are expressed as the means ± standard deviations. We used two-way ANOVA with Tukey's multiple comparison test to assess the effects of noradrenaline treatment on the cell numbers and cAMP concentrations of the A637 and RD sarcoma cancer cell lines. Additionally, we performed an unpaired *t*-test with Holm–Šídák correction to examine the effects of noradrenaline treatment on cell proliferation, and two-way ANOVA with with Šídák's multiple comparison test to analyze the effects on cell migration. The level of significance was set at $p \leq 0.05$ for all tests. GraphPad Prism 9.2.0 (GraphPad, San Diago, CA, USA) was used to visualize the results.

3. Results

To investigate whether differences in adrenergic receptor isoform expressions can explain different cancer hallmark responses to catecholamines, we selected two pediatric sarcoma cancer cell lines that systematically differ in their adrenergic receptor expressions. For this purpose, we retrieved the adrenergic receptor isoform expression data of >1000 cancer cell lines from the cancer cell line encyclopedia [26]. Among sarcoma cancer cell lines, Ewing's sarcoma cell line A673 expresses high amounts of all three groups of adrenergic receptors, with z-transformed expression levels above 4.04 for the α1D-, α2C-, β1-, and β3-adrenergic receptors. In contrast, the RD rhabdomyosarcoma cell line expresses hardly any adrenergic receptors, with z-transformed adrenergic receptor isoform expression levels below 0.4. Both cell lines were authenticated by Eurofins by STR/DNA profiling.

The CCLE adrenergic receptor isoform expression data were confirmed by our RNA sequencing analysis (Figure 1). The treatment with 60 µM of noradrenaline only had minor effects on the adrenergic receptor gene expressions in both the A673 cells and RD cells.

3.1. Effects of Noradrenaline on A673 and RD Sarcoma Cell Numbers

In the first step, we aimed to assess the effects of different noradrenaline concentrations and treatment durations on the cell numbers of A673 and RD sarcoma cells. For this purpose, we treated A673 and RD cells with a 5× dilution series of noradrenaline from 300 µM to 0.096 µM or growth medium (control) and measured the effects of noradrenaline on the cell numbers after 24, 48, and 72 h using a sulforhodamine B (SRB) assay. This showed that noradrenaline concentrations of 0.096, 0.48, 2.4, and 12 µM did not affect cell numbers in both cell lines (Figure 2). In the A673 cells, the treatment with 60 µM of noradrenaline for 72 h decreased the cell numbers significantly by 61.89% ± 10.36 compared to the cells treated with growth medium only. The same treatment did not affect the cell numbers of RD cells. A concentration of 300 µM of noradrenaline decreased the cell numbers by more than 75% after 24 h and by 95% after 48 h in both cell lines (see Supplementary Table S1).

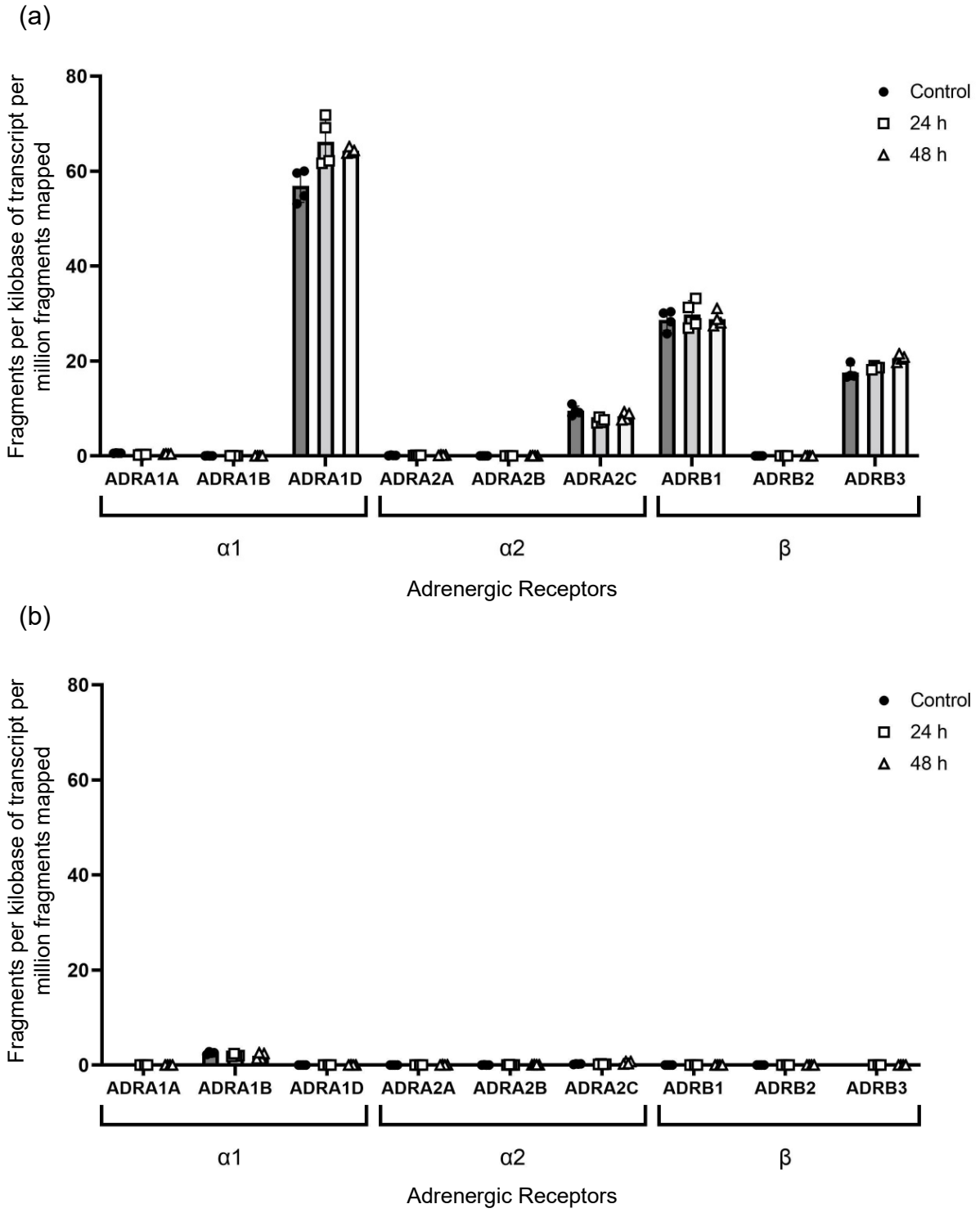

Figure 1. Adrenergic receptor isoform mRNA expressions in (**a**) A673 and (**b**) RD tumor cells. Expressions are displayed as fragments per kilobase of transcript per million fragments mapped (FPKM) in cells treated with 60 μM of noradrenaline and controls treated with growth medium only.

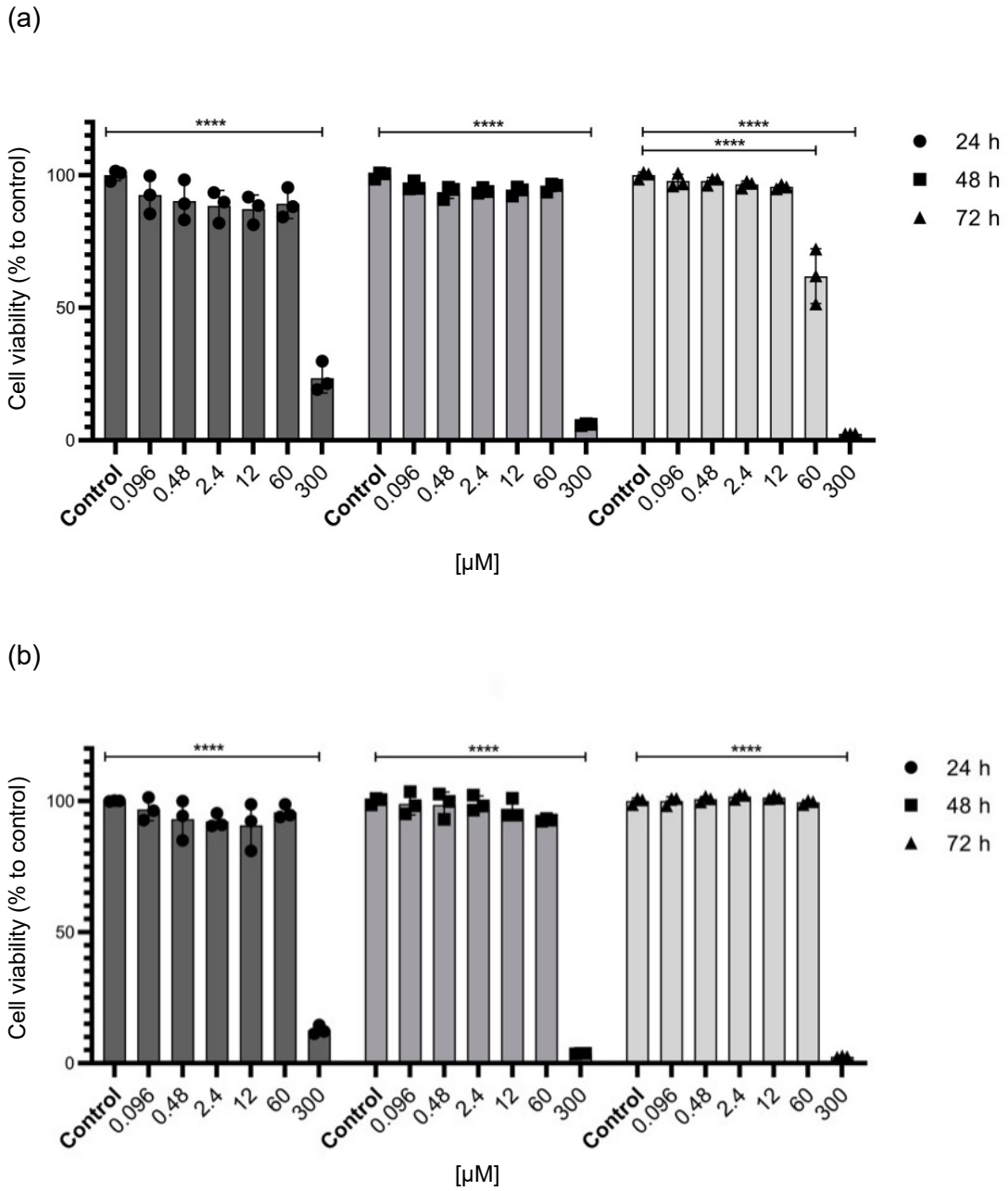

Figure 2. Effects of noradrenaline treatment on cell numbers of (**a**) A673 and (**b**) RD cells after 24 (●), 48 (■) and 72 (▲) hours. While the treatments with 0.096, 0.48, 2.4, and 12 µM of noradrenaline did not affect the cell numbers of either the A673 or the RD sarcoma cells, the treatment with 60 µM of noradrenaline for 72 h significantly increased the cell numbers of the A673 cells but not the RD cells. The treatment of A673 and RD sarcoma cells with 300 µM of noradrenaline for 24 and 48 h significantly reduced the cell numbers of both cell lines. The cell numbers of each sample are illustrated as their percentage compared to the control cells treated with growth medium only. The results are displayed as individual data points as well as the mean ± SD. The asterisks indicate significant differences in the cell numbers between the cells treated with growth medium only (control) and the noradrenaline-treated cells: **** $p \leq 0.001$.

3.2. Effects of Noradrenaline on Proliferation of the A673 and RD Sarcoma Cell Lines

Increased cell proliferation is a hallmark of cancer that contributes to cancer growth and progression. Given the considerable effects of the noradrenaline treatment on the cell numbers of A673 cells, we next aimed to investigate whether this could be associated with

reduced proliferation rates of A673 cells after noradrenaline treatment. For this purpose, we treated A673 and RD sarcoma cells with 60 µM of noradrenaline or growth medium (control) for 72 h and incorporated 5′-ethynyl-2′-deoxyuridine (EdU) into the cells during the last four hours of the treatment period to detect cell proliferation. Additionally, we stained the nuclei using bisBenzimide H 33342 trihydrochloride. Three images per sample were taken with a fluorescence microscope. We counted the cells in three random view fields per image, with more than 2000 cells counted in each experiment. In the A673 cells, the noradrenaline treatment significantly decreased cell proliferation by 15.88% ± 6.76, going from 49.89% ± 5.99 in the control cells to 34.01% ± 1.88 in the cells treated with 60 µM of noradrenaline (Figure 3). The same treatment did not affect the proliferation rates in the RD cells, with 34.20% ± 2.15 proliferating cells in the control and 35.06% ± 4.15 in the treated cells (see Supplementary Table S2).

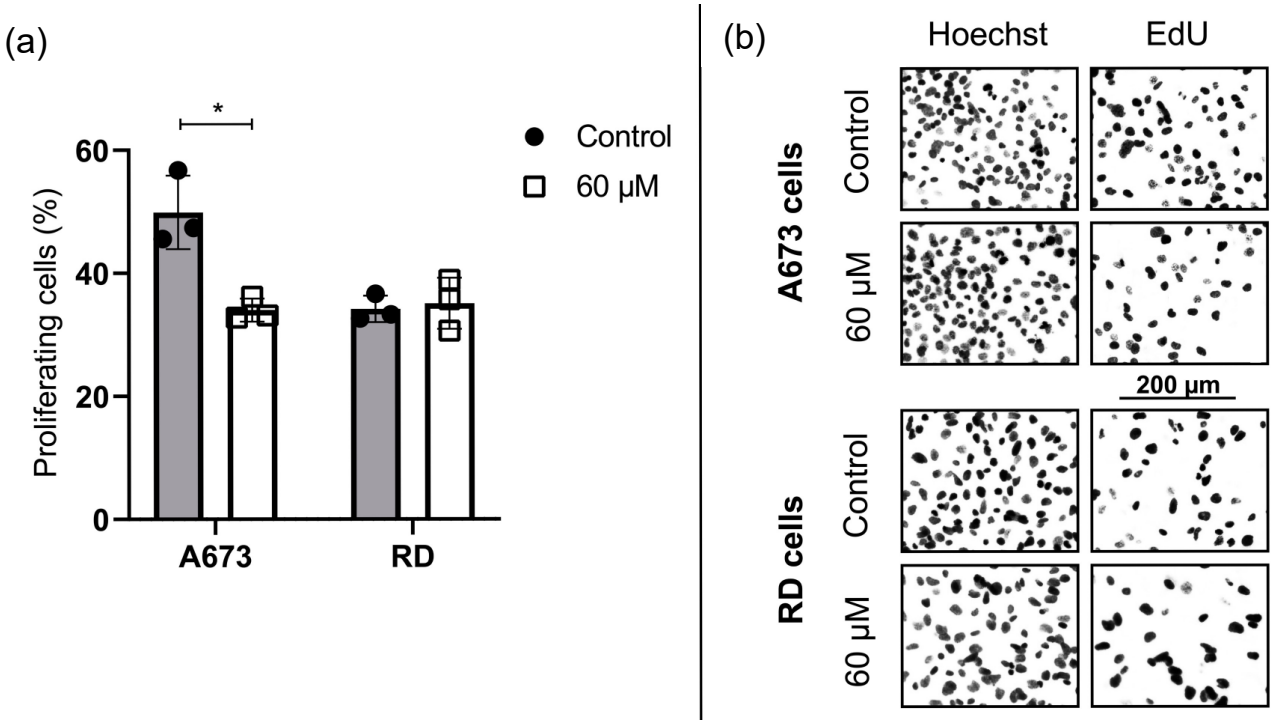

Figure 3. Proliferation rates of A673 and RD sarcoma cells after noradrenaline treatment. (**a**) Treatment with 60 µM of noradrenaline for 72 h significantly decreased cell proliferation of A673 cells, but did not affect cell proliferation rates of RD cells. Asterisks indicate significant differences in cell proliferation between cells treated with 60 µM of noradrenaline (□) and cells treated with growth medium only (•): * $p \leq 0.05$. (**b**) Representative images of fluorescence microscopy view fields of A673 and RD sarcoma cells treated with 60 µM of noradrenaline or growth medium only.

3.3. Effects of Noradrenaline on Cell Migration of A673 and RD Sarcoma Cell Lines

Cell migration is a marker for the metastasis potential of cancer cells. Therefore, we analyzed the effects of noradrenaline on the cell migration of A673 and RD sarcoma cells. We used the ibidi™ migration assay dishes with removable silicone inserts, which leave a 500 µm cell-free gap. The cells were treated with 60 µM of noradrenaline or growth medium (control) for 72 h, and microscopic images of the cell-free gap were captured after 0, 24, 48, and 72 h of treatment. We calculated the percentage of scratch open area using the ibidi wound healing analysis FastTrack AI. This experiment was conducted three times with three technical replicates each. In the A673 cells, the noradrenaline treatment significantly reduced cell migration compared to the cells treated with growth medium only (Figure 4). After 24 h, we observed a remaining scratch open area of 52.47% ± 14.10 in the A673 cells treated with 60 µM of noradrenaline, whereas the control A673 cells had a scratch open area of 25.10% ± 7.64. After 48 h, the A673 cells treated with growth medium

only completely closed the gap, whereas the cells treated with 60 µM of noradrenaline still displayed a scratch open area of 28.59% ± 5.07. In contrast, RD cell migration was not affected by the noradrenaline treatment at any time (see Supplementary Table S3).

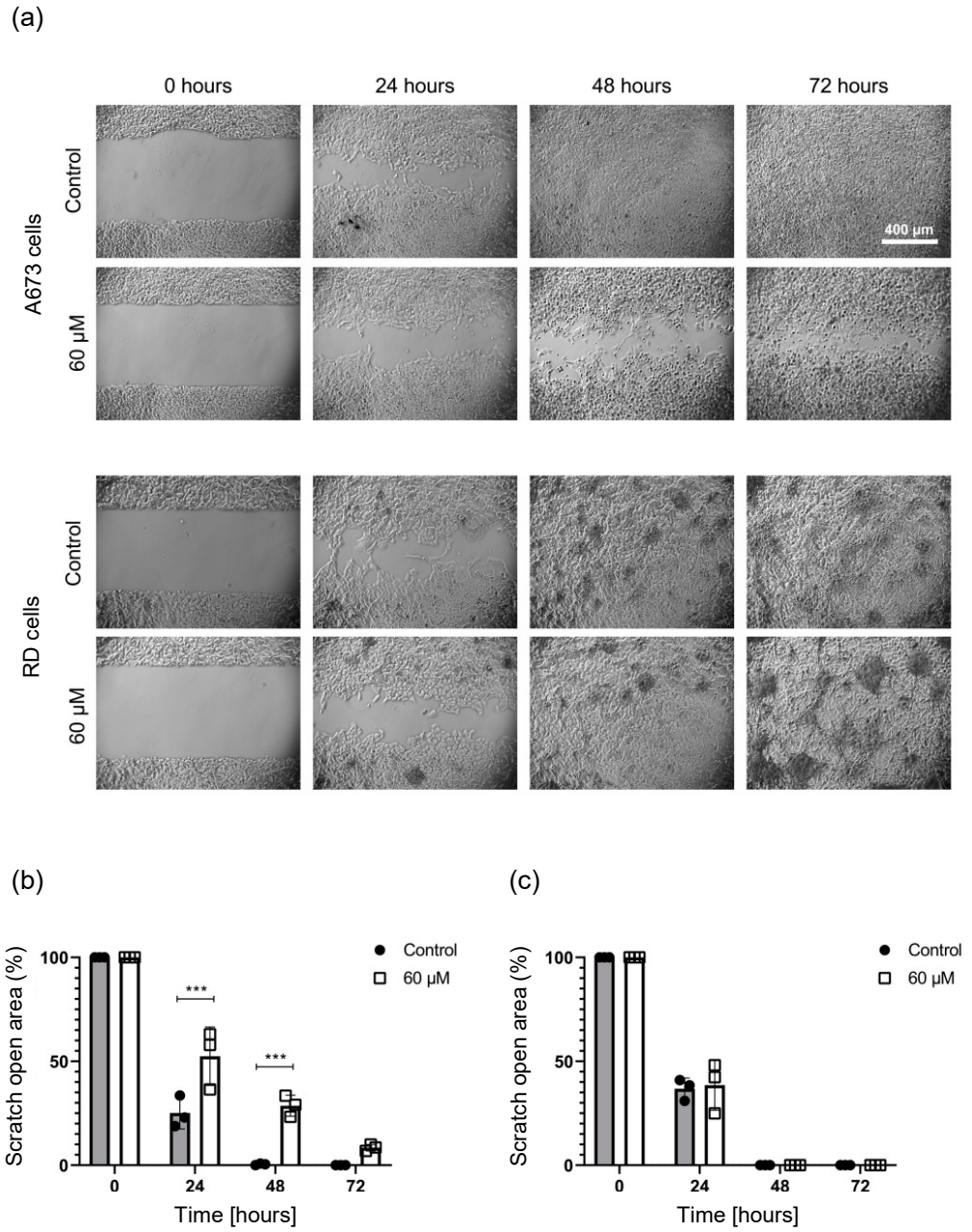

Figure 4. Cell migration of A673 and RD sarcoma cells after noradrenaline treatment. (**a**) Microscopic images of the cell-free gap after 0, 24, 48, and 72 h of treatment with 60 µM noradrenaline or growth medium (control) of A673 and RD sarcoma cells. (**b**) The treatment with 60 µM of noradrenaline for 24 and 48 h significantly decreased cell proliferation of the A673 cells compared to the cells treated with growth medium only (control). (**c**) In the RD cells, the same treatment did not affect cell migration. The results are displayed as individual data points representing one technical replicate each, as well as the mean ± SD of one representative experiment. The asterisks denote significant differences in cell migration between the cells treated with 60 µM of noradrenaline (□) and the cells treated with growth medium only (●): *** $p \leq 0.001$.

3.4. Effects of Noradrenaline on cAMP Signaling in A673 and RD Sarcoma Cell Lines

The cyclic adenosine monophosphate (cAMP) signaling pathway is involved in various aspects of cancer development and progression [29]. We, therefore, aimed to investigate if and how noradrenaline alters the concentration of the second messenger molecule cAMP in A673 and RD sarcoma cells. For this purpose, we measured the cAMP concentrations in A673 and RD cells using a competitive enzyme-linked immunosorbent assay (ELISA) kit after treatment with either 60 µM or 20 nM of noradrenaline or growth medium (control) for 5 or 30 min, respectively. We used at least two technical replicates per sample and three technical replicates in the A673 cells treated with 60 µM of noradrenaline. While the treatment with 60 µM of noradrenaline significantly increased the cAMP levels 38-fold from 0.78 ± 0.13 pmol/mL to 29.15 ± 1.09 pmol/mL after 5 min and to 29.80 ± 1.02 pmol/mL after 30 min in the A673 cells, it did not affect the cAMP concentrations in the RD cells, as expected (Figure 5). Notably, the treatment with a physiological plasma noradrenaline concentration of 20 nM did not affect the cAMP concentrations in either the A673 or the RD cells (see Supplementary Table S4).

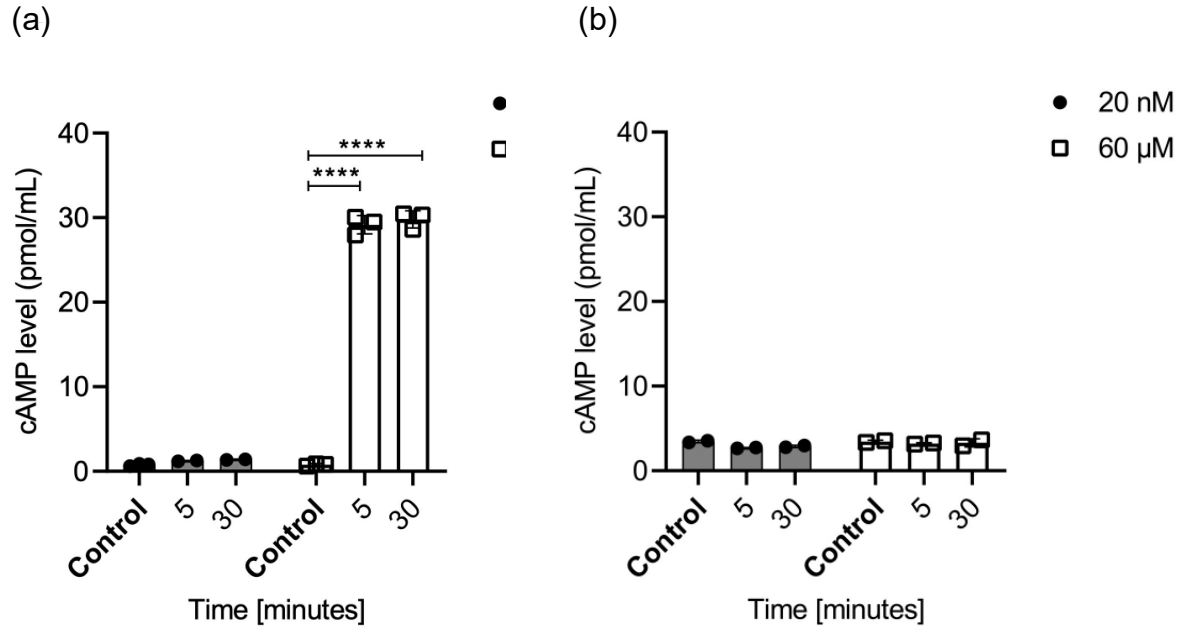

Figure 5. The effects of noradrenaline treatment on the cyclic adenosine monophosphate (cAMP) concentrations in (**a**) A673 and (**b**) RD cells. While the treatment with 60 µM noradrenaline significantly increased the cAMP concentrations in the A673 cells after both 5 and 30 min, it did not affect the cAMP concentrations in the RD cells. The treatment of the A673 and RD sarcoma cells with 20 nM of noradrenaline did not affect the cAMP concentrations in either of the two cell lines. The results are displayed as individual data points representing one technical replicate each, as well as the mean ± SD. The asterisks indicate significant differences in the cAMP concentrations between the cells treated with 60 µM (□) or 20 nM (•) of noradrenaline and the cells treated with growth medium only: **** $p \leq 0.001$.

4. Discussion

Here, we show that A673 and RD sarcoma cells differ in both their adrenergic receptor isoform expressions and their responses to noradrenaline. While A673 cells express α1D-, α2C-, β1-, and β3-adrenergic receptors and respond to noradrenaline treatment with decreased cell proliferation and migration and increased cAMP signaling, RD cells barely express adrenergic receptors and do not respond to noradrenaline. Therefore, the contradictory findings in relation to catecholamine signaling and cancer indeed might be related to differences in the adrenergic receptor isoform expressions.

One of the fundamental characteristics of cancer cells is their ability to proliferate continuously and uncontrollably, even in the absence of external stimuli [30]. Here, we showed that noradrenaline treatment reduced cell numbers in the A673 but not in the RD sarcoma cells (Figure 2), which is likely associated with the reduced proliferation rates of the A673 cells upon noradrenaline treatment (Figure 3). This is in contrast to the results of previous works, where noradrenaline has been shown to enhance the proliferation of gastric [31], breast [32], and colon cancer cells [33]. However, all of these cancer cells express β2-adrenergic receptors, whereas A673 cells mainly express α1D-, α2C-, β1-, and β3-adrenergic receptors, but not β2-adrenergic receptors (z-transformed relative expression of −5.07). Indeed, β2 signaling has been associated with increased cancer cell proliferation before [34]. Consequently, our analyses suggest that noradrenaline might decrease cell proliferation and, hence, delay disease progression in some types of cancers with certain adrenergic receptor isoforms (i.e., α1D-, α2C-, β1-, and/or β3- adrenergic receptors, which are expressed in A673 sarcoma cells), whereas it has detrimental, cancer-promoting effects in others expressing β2-adrenergic receptors.

Another important cancer hallmark besides cell proliferation is the ability of cancer cells to migrate, which is necessary for the development of metastases [35]. While we observed decreased cell migration in the A673 sarcoma cells upon noradrenaline exposure, the RD cells were not affected by the same treatment (Figure 4). In previous works, noradrenaline has been shown to increase migration in breast [36], glioma [37], colon [33], and prostate [38] cancer cells, whereas it decreased migration in glioblastoma [39] and oral [40] cancer cells. In pancreatic cancer cells, noradrenaline has been shown to both increase [41] and decrease [42] cell migration. Again, it is tempting to speculate that β2 signaling might be responsible for the decreased cell migration upon noradrenaline exposure observed in this study, as increased cell migration is associated with β2 signaling [43] and A673 cells do not express β2 receptors. However, cells in oral cancer do express β2-adrenergic receptors and were reported to decrease migration after noradrenaline treatment as well [40]. Further research will be necessary to fully understand the effects of noradrenaline on the metastasis potential of cancer cells and how this might be related to β2-adrenergic receptors.

Cyclic adenosine monophosphate (cAMP) is a second messenger in the cellular response to catecholamines. Depending on the Gα-protein isoform that is coupled to the adrenergic receptor, catecholamine binding can either inhibit or stimulate adenylyl cyclase, which converts ATP to cAMP. Here, we showed that adrenaline significantly increased the cAMP concentrations in the A673 cells but not in the RD sarcoma cells (Figure 5). This is in line with what we know about adrenergic receptors, since A673 cells firmly express β1- (coupled Gα-protein: $G\alpha_S$) and β3- (Gα-protein: $G\alpha_q$) adrenergic receptors, which both stimulate adenyl cyclase and cAMP synthesis upon catecholamine binding [12]. However, the clinical implications of altered cAMP concentrations remain elusive, since cAMP can either suppress or promote cancer cell growth and metastasis depending on the cancer type, stage, and context [44]. What is more, both the agonists and antagonists of cAMP signaling can be used for cancer therapy in a cancer-dependent manner [44]. In A673 cells, the inhibition of the cAMP downstream effector protein kinase A (PKA) has been shown to suppress tumor growth and metastasis [45], which might suggest a tumor-promoting role of cAMP signaling in A673 cells. This is in contrast to the decreased cell proliferation and migration we observed in the A673 cells after the noradrenaline treatment in this study. Future work should aim to selectively express different adrenergic receptor isoforms in receptor null cells, such as RD cells, to see which receptor isoforms mediate which hallmark effects upon noradrenaline stimulation.

Although this study generated some insights into the potential role of adrenergic receptor isoforms on the variable effects of catecholamines in cancer, it still has to be evaluated within the context of its limitations. First and foremost, we analyzed the effects of noradrenaline treatment on different cancer hallmarks in a total of two cell lines only, namely the A673 and RD sarcoma cancer cell lines. These cell lines were chosen based on their contrasting adrenergic receptor expressions, and they represent two of the most com-

mon pediatric sarcomas. Still, it will be essential to confirm our findings in a larger number of cell lines in order to further explore the adrenoreceptor isoform-specific responses of cancer cells to noradrenaline. A second limitation of this study is that it required rather high noradrenaline concentrations to induce cellular responses in vitro. The resting values of unconjugated noradrenaline typically range from 1 to 1.5 nM [25] and can increase up to >20-fold upon maximal exercise [46] up to 25 nM after 10 intervals of 6 s sprints [13]. However, 60 µM of noradrenaline was required to reduce the cell numbers and to increase the cAMP levels in the A673 cells, whereas 20 nM of noradrenaline neither affected the cell numbers nor the cAMP signaling in the A673 and RD sarcoma cells. Consequently, the noradrenaline concentrations necessary to alter cancer hallmarks in vitro might not be reached under physiological conditions in vivo. Again, this should be clarified, e.g., by using exercise-conditioned sera in future in vitro studies, ideally involving a larger number of cancer cell lines. In doing so, future work could also address the third and final limitation of the present study, as we only assessed the effects of noradrenaline treatment on cancer cells and neglected the potential roles of the other catecholamines. This is crucial, since noradrenaline, adrenaline [12], and dopamine [47] release has been shown to affect cancer hallmarks and outcomes. By using exercise-conditioned sera in future studies, it might be possible to mimic the effects of the complex interplay between catecholamines and other co-released factors.

5. Conclusions

This study revealed that the same treatment with noradrenaline induced different proliferation, migration, and cAMP signaling responses in the two sarcoma cell lines A673 and RD. As expected, only the adrenergic receptor-expressing A673 cells responded to the noradrenaline treatment, whereas the non-expressing RD sarcoma cells did not respond. This seems trivial but is a likely mechanism to explain why cancers can respond differently to catecholamine concentration increases due to, e.g., a bout of exercise, a stressful diagnosis, or the stress associated with cancer surgery. In addition, the duration and the magnitude of catecholamine exposure might affect if and how cancer cells respond to exercise- and stress-induced catecholamine release. This might also be linked to the eustress versus chronic stress concept.

While this study provides important insights into the role of adrenergic receptor isoforms in mediating the variable effects of noradrenaline on different cancer hallmarks, further research will be necessary to validate our findings in a larger number of cancer cell lines. This could improve our understanding of the adrenoreceptor isoform-specific responses to noradrenaline treatments across different cancer types, i.e., by using a systematic knock-out or knock-in of specific adrenergic receptor isoforms alone or in combination. Additionally, the relatively high noradrenaline concentrations required to elicit a response in vitro highlight the need for future studies to investigate the impact of more physiologically relevant conditions, such as using exercise-conditioned sera, to better mimic the in vivo environment. This approach would also allow for the exploration of the effects of other catecholamines, like adrenaline and dopamine, which play crucial roles in cancer biology but were not addressed in this present study. By broadening the scope of research to include these factors and conducting more comprehensive in vitro experiments, future work could provide a more holistic understanding of the complex interactions between catecholamines and cancer cells. This may help to further personalize cancer treatments in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/onco4040028/s1, Table S1: Effect of noradrenaline on A673 and RD sarcoma cell numbers; Table S2: Effect of noradrenaline on cell proliferation in A673 and RD sarcoma cell lines; Table S3: Effect of noradrenaline on cell migration in A673 and RD sarcoma cell lines; Table S4: Effect of noradrenaline on cAMP signaling in A673 and RD sarcoma cell lines.

Author Contributions: Conceptualization: H.W. and I.v.L.; formal analysis: P.W., J.H. and C.R.; funding acquisition: H.W. and I.v.L.; investigation: P.W. and J.H.; methodology: P.W., J.H. and M.S.; project administration: H.W. and I.v.L.; supervision: M.S., H.W., S.B. and I.v.L.; visualization: P.W.; writing—original draft: P.W.; writing—review and editing: S.B., C.R., M.S. and H.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Zukunft Gesundheit e.V.; Funding number: 8810003072.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated during this study are available in the Supplementary Materials.

Conflicts of Interest: All authors declare no conflicts of interest.

Abbreviations

cAMP: cyclic adenosine monophosphate; CCLE: cancer cell encyclopedia; EdU: 5′-ethynyl-2′-deoxyuridine; ELISA: enzyme-linked immunosorbent assay; FBS: fetal bovine serum; FPKM: fragments per kilobase of transcripts per million fragments mapped; GPCR: G protein-coupled receptor; HCl: hydrogen chloride; PBS: phosphate-buffered saline; SRB: sulforhodamine B.

References

1. Moore, S.C.; Lee, I.M.; Weiderpass, E.; Campbell, P.T.; Sampson, J.N.; Kitahara, C.M.; Keadle, S.K.; Arem, H.; De Gonzalez, A.B.; Hartge, P.; et al. Association of Leisure-Time Physical Activity with Risk of 26 Types of Cancer in 1.44 Million Adults. *JAMA Intern. Med.* **2016**, *176*, 816–825. [CrossRef] [PubMed]
2. Betof, A.S.; Dewhirst, M.W.; Jones, L.W. Effects and Potential Mechanisms of Exercise Training on Cancer Progression: A Translational Perspective. *Brain Behav. Immun.* **2013**, *30*, S75–S87. [CrossRef]
3. McTiernan, A.N.N.E.; Friedenreich, C.M.; Katzmarzyk, P.T.; Powell, K.E.; Macko, R.; Buchner, D.; Pescatello, L.S.; Bloodgood, B.; Tennant, B.; Vaux-Bjerke, A.; et al. Physical Activity in Cancer Prevention and Survival: A Systematic Review. *Med. Sci. Sports Exerc.* **2019**, *51*, 1252–1261. [CrossRef] [PubMed]
4. Stout, N.L.; Baima, J.; Swisher, A.K.; Winters-Stone, K.M.; Welsh, J. A Systematic Review of Exercise Systematic Reviews in the Cancer Literature (2005–2017). *PM&R* **2017**, *9*, S347–S384.
5. Christensen, J.F.; Simonsen, C.; Hojman, P. Exercise Training in Cancer Control and Treatment. *Compr. Physiol.* **2018**, *9*, 165–205.
6. Rock, C.L.; Thomson, C.; Gansler, T.; Gapstur, S.M.; McCullough, M.L.; Patel, A.V.; Andrews, K.S.; Bandera, E.V.; Spees, C.K.; Robien, K.; et al. American Cancer Society Guideline for Diet and Physical Activity for Cancer Prevention. *CA Cancer J. Clin.* **2020**, *70*, 245–271. [CrossRef]
7. Patel, A.V.; Friedenreich, C.M.; Moore, S.C.; Hayes, S.C.; Silver, J.K.; Campbell, K.L.; Winters-Stone, K.; Gerber, L.H.; George, S.M.; Fulton, J.E.; et al. American College of Sports Medicine Roundtable Report on Physical Activity, Sedentary Behavior, and Cancer Prevention and Control. *Med. Sci. Sports Exerc.* **2019**, *51*, 2391–2402. [CrossRef]
8. Leal, L.G.; Lopes, M.A.; Batista, M.L., Jr. Physical Exercise-Induced Myokines and Muscle-Adipose Tissue Crosstalk: A Review of Current Knowledge and the Implications for Health and Metabolic Diseases. *Front. Physiol.* **2018**, *9*, 1307. [CrossRef]
9. Schranner, D.; Schonfelder, M.; Romisch-Margl, W.; Scherr, J.; Schlegel, J.; Zelger, O.; Riermeier, A.; Kaps, S.; Prehn, C.; Adamski, J.; et al. Physiological Extremes of the Human Blood Metabolome: A Metabolomics Analysis of Highly Glycolytic, Oxidative, and Anabolic Athletes. *Physiol. Rep.* **2021**, *9*, e14885. [CrossRef]
10. Idorn, M.; Hojman, P. Exercise-Dependent Regulation of Nk Cells in Cancer Protection. *Trends Mol. Med.* **2016**, *22*, 565–577. [CrossRef]
11. Copeland, J.L.; Consitt, L.A.; Tremblay, M.S. Hormonal Responses to Endurance and Resistance Exercise in Females Aged 19–69 Years. *J. Gerontol. A Biol. Sci. Med. Sci.* **2002**, *57*, B158–B165. [CrossRef] [PubMed]
12. Wackerhage, H.; Christensen, J.F.; Ilmer, M.; von Luettichau, I.; Renz, B.W.; Schonfelder, M. Cancer Catecholamine Conundrum. *Trends Cancer* **2022**, *8*, 110–122. [CrossRef] [PubMed]
13. Bracken, R.M.; Linnane, D.M.; Brooks, S. Plasma Catecholamine and Nephrine Responses to Brief Intermittent Maximal Intensity Exercise. *Amino Acids* **2009**, *36*, 209–217. [CrossRef] [PubMed]
14. Pedersen, L.; Idorn, M.; Olofsson, G.H.; Lauenborg, B.; Nookaew, I.; Hansen, R.H.; Johannesen, H.H.; Becker, J.C.; Pedersen, K.S.; Dethlefsen, C.; et al. Voluntary Running Suppresses Tumor Growth through Epinephrine- and Il-6-Dependent Nk Cell Mobilization and Redistribution. *Cell Metab.* **2016**, *23*, 554–562. [CrossRef]
15. Liu, C.; Yang, Y.; Chen, C.; Li, L.; Li, J.; Wang, X.; Chu, Q.; Qiu, L.; Ba, Q.; Li, X.; et al. Environmental Eustress Modulates Beta-Ars/Ccl2 Axis to Induce Anti-Tumor Immunity and Sensitize Immunotherapy against Liver Cancer in Mice. *Nat. Commun.* **2021**, *12*, 5725. [CrossRef]

16. Yu, F.X.; Zhao, B.; Panupinthu, N.; Jewell, J.L.; Lian, I.; Wang, L.H.; Zhao, J.; Yuan, H.; Tumaneng, K.; Li, H.; et al. Regulation of the Hippo-Yap Pathway by G-Protein-Coupled Receptor Signaling. *Cell* **2012**, *150*, 780–791. [CrossRef]
17. Goldstein, D.S. Catecholamines and Stress. *Endocr. Regul.* **2003**, *37*, 69–80.
18. Altosaar, K.; Balaji, P.; Bond, R.A.; Bylund, D.B.; Cotecchia, S.; Devost, D.; Doze, V.A.; Eikenburg, D.C.; Gora, S.; Goupil, E.; et al. Adrenoceptors (Version 2019.4) in the Iuphar/Bps Guide to Pharmacology Database. *IUPHAR/BPS Guide Pharmacol. CITE* **2019**, *2019*, 4. [CrossRef]
19. Dethlefsen, C.; Hansen, L.S.; Lillelund, C.; Andersen, C.; Gehl, J.; Christensen, J.F.; Pedersen, B.K.; Hojman, P. Exercise-Induced Catecholamines Activate the Hippo Tumor Suppressor Pathway to Reduce Risks of Breast Cancer Development. *Cancer Res.* **2017**, *77*, 4894–4904. [CrossRef]
20. Cui, B.; Luo, Y.; Tian, P.; Peng, F.; Lu, J.; Yang, Y.; Su, Q.; Liu, B.; Yu, J.; Luo, X.; et al. Stress-Induced Epinephrine Enhances Lactate Dehydrogenase a and Promotes Breast Cancer Stem-Like Cells. *J. Clin. Investig.* **2019**, *129*, 1030–1046. [CrossRef]
21. Renz, B.W.; Takahashi, R.; Tanaka, T.; Macchini, M.; Hayakawa, Y.; Dantes, Z.; Maurer, H.C.; Chen, X.; Jiang, Z.; Westphalen, C.B.; et al. Beta2 Adrenergic-Neurotrophin Feedforward Loop Promotes Pancreatic Cancer. *Cancer Cell* **2018**, *34*, 863–867. [CrossRef] [PubMed]
22. Na, Z.; Qiao, X.; Hao, X.; Fan, L.; Xiao, Y.; Shao, Y.; Sun, M.; Feng, Z.; Guo, W.; Li, J.; et al. The Effects of Beta-Blocker Use on Cancer Prognosis: A Meta-Analysis Based on 319,006 Patients. *Onco Targets Ther.* **2018**, *11*, 4913–4944. [CrossRef] [PubMed]
23. Yap, A.; Lopez-Olivo, M.A.; Dubowitz, J.; Pratt, G.; Hiller, J.; Gottumukkala, V.; Sloan, E.; Riedel, B.; Schier, R. Effect of Beta-Blockers on Cancer Recurrence and Survival: A Meta-Analysis of Epidemiological and Perioperative Studies. *Br. J. Anaesth.* **2018**, *121*, 45–57. [CrossRef] [PubMed]
24. Hein, L.; Kobilka, B.K. Adrenergic Receptor Signal Transduction and Regulation. *Neuropharmacology* **1995**, *34*, 357–366. [CrossRef] [PubMed]
25. Tank, A.W.; Wong, D.L. Peripheral and Central Effects of Circulating Catecholamines. *Compr. Physiol.* **2015**, *5*, 1–15.
26. Barretina, J.; Caponigro, G.; Stransky, N.; Venkatesan, K.; Margolin, A.A.; Kim, S.; Wilson, C.J.; Lehar, J.; Kryukov, G.V.; Sonkin, D.; et al. The Cancer Cell Line Encyclopedia Enables Predictive Modelling of Anticancer Drug Sensitivity. *Nature* **2012**, *483*, 603–607. [CrossRef]
27. Ghandi, M.; Huang, F.W.; Jané-Valbuena, J.; Kryukov, G.V.; Lo, C.C.; McDonald, E.R., III; Barretina, J.; Gelfand, E.T.; Bielski, C.M.; Li, H.; et al. Next-Generation Characterization of the Cancer Cell Line Encyclopedia. *Nature* **2019**, *569*, 503–508. [CrossRef]
28. Vichai, V.; Kirtikara, K. Sulforhodamine B Colorimetric Assay for Cytotoxicity Screening. *Nat. Protoc.* **2006**, *1*, 1112–1116. [CrossRef]
29. Ahmed, M.B.; Alghamdi, A.A.A.; Islam, S.U.; Lee, J.S.; Lee, Y.S. Camp Signaling in Cancer: A Pka-Creb and Epac-Centric Approach. *Cells* **2022**, *11*, 2020. [CrossRef]
30. Gutschner, T.; Diederichs, S. The Hallmarks of Cancer: A Long Non-Coding Rna Point of View. *RNA Biol.* **2012**, *9*, 703–719. [CrossRef]
31. Zhi, X.; Li, B.; Li, Z.; Zhang, J.; Yu, J.; Zhang, L.; Xu, Z. Adrenergic Modulation of Ampk-Dependent Autophagy by Chronic Stress Enhances Cell Proliferation and Survival in Gastric Cancer. *Int. J. Oncol.* **2019**, *54*, 1625–1638. [CrossRef] [PubMed]
32. Zhou, J.; Liu, Z.; Zhang, L.; Hu, X.; Wang, Z.; Ni, H.; Wang, Y.; Qin, J. Activation of Beta2-Adrenergic Receptor Promotes Growth and Angiogenesis in Breast Cancer by Down-Regulating Ppargamma. *Cancer Res. Treat.* **2020**, *52*, 830–847. [CrossRef] [PubMed]
33. Han, J.; Jiang, Q.; Ma, R.; Zhang, H.; Tong, D.; Tang, K.; Wang, X.; Ni, L.; Miao, J.; Duan, B.; et al. Norepinephrine-Creb1-Mir-373 Axis Promotes Progression of Colon Cancer. *Mol. Oncol.* **2020**, *14*, 1059–1073. [CrossRef] [PubMed]
34. Kaira, K.; Kamiyoshihara, M.; Kawashima, O.; Endoh, H.; Imaizumi, K.; Sugano, M.; Tanaka, S.; Fujita, A.; Kogure, Y.; Shimizu, A.; et al. Prognostic Impact of Beta2 Adrenergic Receptor Expression in Surgically Resected Pulmonary Pleomorphic Carcinoma. *Anticancer Res.* **2019**, *39*, 395–403. [CrossRef]
35. Chaffer, C.L.; Weinberg, R.A. A Perspective on Cancer Cell Metastasis. *Science* **2011**, *331*, 1559–1564. [CrossRef]
36. Drell, T.; Joseph, J.; Lang, K.; Niggemann, B.; Zaenker, K.S.; Entschladen, F. Effects of Neurotransmitters on the Chemokinesis and Chemotaxis of Mda-Mb-468 Human Breast Carcinoma Cells. *Breast Cancer Res. Treat.* **2003**, *80*, 63–70. [CrossRef]
37. Wang, X.; Wang, Y.; Xie, F.; Song, Z.T.; Zhang, Z.Q.; Zhao, Y.; Wang, S.D.; Hu, H.; Zhang, Y.S.; Qian, L.J. Norepinephrine Promotes Glioma Cell Migration through up-Regulating the Expression of Twist1. *BMC Cancer* **2022**, *22*, 213. [CrossRef]
38. Barbieri, A.; Bimonte, S.; Palma, G.; Luciano, A.; Rea, D.; Giudice, A.; Scognamiglio, G.; La Mantia, E.; Franco, R.; Perdona, S.; et al. The Stress Hormone Norepinephrine Increases Migration of Prostate Cancer Cells in Vitro and in Vivo. *Int. J. Oncol.* **2015**, *47*, 527–534. [CrossRef]
39. Zhong, J.; Shan, W.; Zuo, Z. Norepinephrine Inhibits Migration and Invasion of Human Glioblastoma Cell Cultures Possibly Via Mmp-11 Inhibition. *Brain Res.* **2021**, *1756*, 147280. [CrossRef]
40. Bravo-Calderon, D.M.; Assao, A.; Garcia, N.G.; Coutinho-Camillo, C.M.; Roffe, M.; Germano, J.N.; Oliveira, D.T. Beta Adrenergic Receptor Activation Inhibits Oral Cancer Migration and Invasiveness. *Arch. Oral Biol.* **2020**, *118*, 104865. [CrossRef]
41. Huang, X.Y.; Wang, H.C.; Yuan, Z.; Huang, J.; Zheng, Q. Norepinephrine Stimulates Pancreatic Cancer Cell Proliferation, Migration and Invasion Via Beta-Adrenergic Receptor-Dependent Activation of P38/Mapk Pathway. *Hepatogastroenterology* **2012**, *59*, 889–893. [PubMed]
42. Stock, A.M.; Powe, D.G.; Hahn, S.A.; Troost, G.; Niggemann, B.; Zanker, K.S.; Entschladen, F. Norepinephrine Inhibits the Migratory Activity of Pancreatic Cancer Cells. *Exp. Cell Res.* **2013**, *319*, 1744–1758. [CrossRef]

43. Gruet, M.; Cotton, D.; Coveney, C.; Boocock, D.J.; Wagner, S.; Komorowski, L.; Rees, R.C.; Pockley, A.G.; Garner, A.C.; Wallis, J.D.; et al. Beta2-Adrenergic Signalling Promotes Cell Migration by Upregulating Expression of the Metastasis-Associated Molecule Lypd3. *Biology* **2020**, *9*, 39. [CrossRef] [PubMed]
44. Zhang, H.; Liu, Y.; Liu, J.; Chen, J.; Wang, J.; Hua, H.; Jiang, Y. Camp-Pka/Epac Signaling and Cancer: The Interplay in Tumor Microenvironment. *J. Hematol. Oncol.* **2024**, *17*, 5. [CrossRef] [PubMed]
45. Luo, W.; Xu, C.; Ayello, J.; Cruz, F.D.; Rosenblum, J.M.; Lessnick, S.L.; Cairo, M.S. Protein Phosphatase 1 Regulatory Subunit 1a in Ewing Sarcoma Tumorigenesis and Metastasis. *Oncogene* **2018**, *37*, 798–809. [CrossRef]
46. Zouhal, H.; Jacob, C.; Delamarche, P.; Gratas-Delamarche, A. Catecholamines and the Effects of Exercise, Training and Gender. *Sports Med.* **2008**, *38*, 401–423. [CrossRef]
47. Grant, C.E.; Flis, A.L.; Ryan, B.M. Understanding the Role of Dopamine in Cancer: Past, Present and Future. *Carcinogenesis* **2022**, *43*, 517–527. [CrossRef]