J Physiol 0.0 (2020) pp 1–19

The Journal of Physiology

Physical activity and advanced cancer: evidence of exercise-sensitive genes regulating prostate cancer cell proliferation and apoptosis

Raphaela Schwappacher[1,2], Kristin Schink[1,2], Svetlana Sologub[1,2], Walburga Dieterich[1,2], Dejan Reljic[1,2], Oliver Friedrich[3], Hans J. Herrmann[1,2], Markus F. Neurath[1] and Yurdagül Zopf[1,2]

[1]*Medical Department 1, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany*
[2]*Hector-Centre for Nutrition, Exercise and Sports, Medical Department 1, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany*
[3]*Institute of Medical Biotechnology, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany*

Edited by: Laura Bennet & Russell Hepple

Key points

- Physical activity is known to protect against cancer.
- The resistance exercise method whole-body electromyostimulation (WB-EMS) has a significant anti-cancer effect.
- WB-EMS-conditioned serum from advanced prostate cancer patients decreased human prostate carcinoma cell growth and viability *in vitro*.
- Multiplex analysis revealed that genes associated with human prostate cancer cell proliferation and apoptosis are sensitive for exercise.
- Feasible exercise should be part of multimodal anti-cancer therapies, also for physically weakened patients.

Abstract Regular physical activity is known to protect against cancer development. In cancer survivors, exercise reduces the risk of cancer recurrence and mortality. However, the link between exercise and decreased cancer risk and improved survival is still not well understood. Serum from exercising healthy individuals inhibits proliferation and activates apoptosis in various cancer cells, suggesting that mechanisms regulating cancer cell growth are affected by exercise. For the first time, we analysed serum from advanced-stage cancer patients with prostate (exercise group $n = 8$; control group $n = 10$) or colorectal (exercise $n = 6$; control $n = 6$) cancer, after a 12-week whole-body electromyostimulation training (20 min/session, 2×/week; frequency 85 Hz; pulse width 350 µs; 6 s stimulation, 4 s rest), a tolerable, yet effective, resistance exercise for physically weakened patients. We report that serum from these advanced cancer patients inhibits proliferation and enhances apoptosis of human prostate and colon cancer cells *in vitro* using cell growth and death assays (5-bromo-2′-deoxyuridine incorporation, cell counting, DNA fragmentation). Exercise-mimicking electric pulse stimulation of human primary myotubes showed that electric pulse stimulation-conditioned myotube medium also impairs human cancer cell viability. Gene expression analysis using a multiplex array of cancer-associated genes and subsequent quantitative RT-PCR revealed the presence of exercise-sensitive genes in human prostate cancer cells that potentially participate in the exercise-mediated regulation of malignant cell growth and apoptosis. Our data document the strong efficiency of the anti-oncogenic effects of physical activity and will further support the application of regular therapeutic exercise during cancer disease.

R. Schwappacher and K. Schink contributed equally.

DOI: 10.1113/JP279150

(Received 22 October 2019; accepted after revision 6 July 2020; first published online 9 July 2020)
Corresponding author Y. Zopf: Hector-Centre for Nutrition, Exercise and Sports, Ulmenweg 18, 91054 Erlangen, Germany. Email: yurdaguel.zopf@uk-erlangen.de

Introduction

A sedentary lifestyle and overweight are significant risk factors for cancer. Regular exercise is known to protect against the development of cancer, and in cancer survivors, exercise reduces the risk of cancer recurrence and mortality (Hojman *et al.* 2011; Goh *et al.* 2014; Brown & Gilmore, 2020). Moreover, it is now widely acknowledged that cancer patients can safely participate in adapted exercise programmes and that physical activity is an important supportive part of a cancer treatment (Schwartz *et al.* 2017). Exercise effects in the body are complex and our understanding of how exercise-triggered mechanisms influence cancer development and/or progression is incomplete. Diverse indirect mechanisms are partially responsible for exercise benefits against cancer, e.g. exercise-induced decrease of chronic inflammation (Goh *et al.* 2014; Dieli-Conwright *et al.* 2018). In recent years, researchers found significant evidence that skeletal muscle activation directly affects malignant cell growth. Hojman *et al.* (2011) showed, for the first time, that conditioned serum from exercising mice decreases human breast cancer cell growth *in vitro*. Further, several *in vivo* and *in vitro* studies suggested that serum from healthy humans or mice after acute or long-term exercise attenuates proliferation and activates apoptosis of human cancer cells (Ngo *et al.* 2002; Aoi *et al.* 2013; Rundqvist *et al.* 2013). Dethlefsen *et al.* (2017) found in a comparative approach that serum from healthy women and patients with stage I/II breast cancer after acute exercise has similar inhibitory effects on cancer cell culture growth. Recently, it was shown that serum from colon cancer survivors after acute high intensity aerobic exercise reduces cancer cell growth *in vitro* (Devin *et al.* 2019). However, very little is known about the impact of exercise-conditioned serum from advanced-stage cancer patients on malignant cell proliferation and apoptosis. We hypothesized that the anti-cancer effects of exercise also occur in physically weakened, advanced cancer patients, in part cachectic with a deteriorating muscular status. It is recommended that cancer patients should regularly perform resistance training in order to counteract the loss of muscle mass and strength (Schwartz *et al.* 2017). But traditional resistance training, commonly conducted with weights, can be too strenuous for advanced cancer patients due to poor physical condition or even contra-indicated, e.g. in the presence of bone metastases.

In 2018, we published that whole-body electromyostimulation (WB-EMS) training is feasible, well tolerated and effective in improving body composition and function in advanced-stage cancer patients with solid tumours or haematological malignancies under curative and palliative therapy (Schink *et al.* 2018*a*,*b*). WB-EMS is an innovative, time-efficient form of resistance exercise. It is a joint-friendlier, less exhausting exercise training, and thus can be a reasonable alternative, for example, for patients with physical limitations. The electrical muscle stimulation was applied by bipolar impulses at a frequency of 85 Hz and a pulse width of 350 µs in bouts of 6 s stimulation and 4 s resting intervals (in 20 min sessions, twice a week), which allowed the simultaneous activation of all major skeletal muscle groups (Kemmler *et al.* 2010; Kemmler and von Stengel, 2013; Schink *et al.* 2018*a*). The current intensity during WB-EMS is set to generate a noticeable but comfortable muscle contraction. The current intensity is individually adjusted for each patient, ideally between insufficient and excess training load, in order to achieve the optimal exercise intensity (Filipovic *et al.* 2012; von Stengel *et al.* 2012). There is profound evidence that physical training directly affects cancer cells via serum factors; this was demonstrated in cell culture experiments using exercise-conditioned serum from healthy humans and mice (Hojman *et al.* 2011; Aoi *et al.* 2013; Rundqvist *et al.* 2013). Our research objectives for the present *in vitro* study were (1) to learn more about the anti-cancer effects of exercise in advanced cancer patients, and (2) to examine, for the first time, the effectiveness of the exercise method WB-EMS on malignant cell growth and viability. To address these research aims, we used cancer cell cultures in order to analyse WB-EMS serum from stage III/IV cancer patients with prostate carcinomas, a hormone-dependent type of cancer, or with colorectal carcinomas. In contrast to non-exercising cancer patients, our *in vitro* studies indicated that post-WB-EMS serum decreases human cancer cell viability and, as a potential underlying mechanism, suggested that exercise-sensitive genes are involved in the regulation of proliferation and apoptosis of human prostate cancer cells.

Methods

To analyse the effect of exercise-conditioned serum on human cancer cell growth *in vitro*, we used serum collected from advanced-stage prostate and colorectal cancer patients, who either underwent exercise via WB-EMS training or acted as non-exercising controls (Schink *et al.* 2018*a*).

Ethical approval

The original study was conducted according to the guidelines of the *Declaration of Helsinki*. The study protocol was approved by the ethics committee of the Friedrich-Alexander-University Erlangen-Nürnberg (Reg. No.155_13B) and is registered at clinicaltrials.gov (NCT02293239). Informed, written consent was obtained from the participants.

Clinical study design, patient recruitment and WB-EMS exercise intervention

Patient recruitment and design of the original study (conducted from 2014 to 2017) are described in detail in Schink *et al.* (2018*a*). Briefly, patients (≥18 years old), who were diagnosed with a stage III/IV solid tumour disease and with ongoing anti-cancer therapy, and a Karnofsky performance index between 100% and 60%, were included. After baseline assessment, patients were allocated to an exercise group performing WB-EMS training over a period of 12 weeks or to a control group without WB-EMS. Both groups received nutritional counselling during the study to achieve a daily protein intake of >1.0 g kg^{-1} body weight, and patients with disease-related decreased food intake received medical nutritional therapy. The total energy and nutrient intake of the patients was determined as described in Schink *et al.* (2018*a*), and their individual nutritional intake was monitored throughout the study. The group with WB-EMS performed two training sessions per week for 12 weeks. The patients were adapted to WB-EMS by an incremental increase of the training duration up to 20 min/training session. WB-EMS applies low-frequency current impulses (<100 Hz) with a low current intensity (<100 mA) via electrodes (Kemmler *et al.* 2012). The patients wore a vest, a hip belt, and upper-arm and -thigh cuffs with integrated electrodes (miha bodytec GmbH, Gersthofen, Germany). Eight muscle groups were addressed by the WB-EMS application (upper arms, chest, upper back, latissimus, abdomen, lower back, buttocks and thighs; Fig. 1*A*). Electric stimulation was mediated by bipolar impulses (frequency 85 Hz, pulse width 350 µs), inducing an intermittent stimulation of a 6 s impulse phase followed by 4 s rest. WB-EMS is classified as a resistance exercise. The current intensity was set to trigger a noticeable muscle contraction. Then, the current intensity was raised up to a threshold between being comfortable and inducing discomfort/pain. The current intensity on the miha bodytec device (Fig. 1*A*) is set via a main regulator (setting for our prostate cancer patients between 70 and 75) and the regulators for each muscle group. During the initial adaption phase, the following settings were applied (data for 6 WB-EMS prostate cancer patients): regulator for the leg electrodes 18–49; buttock 10–61; lower back 19–39; middle back 16–41; upper back 19–47; abdomen 16–51; breast 18–53; arm 14–35. After the initial adaption to the training, the following settings were applied (data for 4 WB-EMS prostate cancer patients): leg 41–64; buttock 28–79; lower back 23–47; middle back 24–46; upper back 21–45; abdomen 28–51; breast 33–72; arm 23–44). The individual and local adjustments are highly relevant due to each subject's current tolerance limits and differences in subcutaneous fat thickness (Kemmler *et al.* 2012). Our prostate cancer patients in the intervention group performed 20.75 ± 2.61 WB-EMS training sessions out of the scheduled 24 sessions, and our colorectal WB-EMS cancer patients attended 20.5 ± 2.43 training sessions. The WB-EMS training was individually supervised by experienced physiotherapists and included light physical exercises (for more details, refer to Schink *et al.* 2018*a*).

Patient serum

Blood samples for cancer cell stimulation were collected at the beginning of the trial and at trial end 1 h after the last training session. Blood samples were centrifuged and the serum was stored at −80°C. For the later designed *in vitro* study, we used serum from stage III/IV prostate (exercise group $n = 8$, control group $n = 10$) and stage III/IV colorectal cancer patients (exercise group $n = 6$, control group $n = 6$) under anti-cancer therapy (see Table 1). Serum from 6 out of 10 control prostate cancer patients was obtained during therapy-relevant attendance at the outpatient uro-oncological unit, Medical Department 5, University Clinic Erlangen. The human serum used in electric pulse stimulation (EPS) assays was a serum pool from 12 healthy individuals (6 male, 6 female), who were not associated with the clinical study; their average age was 34.2 ± 10.5 years.

Cell culture

The human prostate cancer cell line LNCaP was cultured in RPMI without phenol red (Thermo Fisher Scientific, Waltham, MA, USA) plus 10% fetal calf serum (FCS) Superior (Biochrom, Merck KGaA, Darmstadt, Germany), 100 IU ml^{-1} penicillin (Biochrom) and 100 µg ml^{-1} streptomycin (Biochrom) at 37°C. The cell line was a kind gift from R. Atreya, Medical Department 1, Friedrich-Alexander-University Erlangen-Nürnberg (RRID:CVCL_0395; experiments were performed in passages 5–25 after purchase). The human prostate cancer cell line DU145 was cultured in RPMI without phenol red, 10% FCS Superior and penicillin–streptomycin at 37°C. The cell line was a kind gift from B. Wullich, Department of Urology, Friedrich-Alexander-University Erlangen-Nürnberg (RRID:CVCL_0105; cells were kept in culture for no longer than 20 passages). The

human prostate cancer cell line PC3 was cultured in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific), 10% FCS Superior and penicillin–streptomycin at 37°C (RRID:CVCL_0035; cells were kept in culture for no longer than 30 passages). The human colon cancer cell line HT29 was maintained in DMEM, 10% FCS Superior and penicillin–streptomycin at 37°C. The cell line was a kind gift from R. Lopez-Posados, Medical Department 1, Friedrich-Alexander-University Erlangen-Nürnberg (RRID:CVCL_0320; experiments were performed in passages 15–30 after purchase). The human embryonic kidney cells HEK293T were maintained in DMEM, 10% FCS Superior and penicillin–streptomycin at 37°C. The cell line was a kind gift from A. Kremer, Medical Department 1, Friedrich-Alexander-University Erlangen-Nürnberg (RRID:CVCL_0045; cells were kept in culture for no longer than 30 passages). Primary human skeletal muscle myoblasts (HSMMs, Lonza, Basel, Switzerland) were cultured according to manufacturer's instructions at 37°C. All cell cultures were mycoplasma-free.

Human cancer cell growth and apoptosis assays

Prostate (LNCaP, DU145, PC3), colon (HT29) cancer cells or non-malignant cells (HEK293T) were seeded into 96-well plates (3–5 $\times$ 10^3 cells per well) or 48-well plates (2.5 $\times$ 10^4 cells per well), and after overnight incubation at 37°C, cells were serum-starved (0.1% FCS) for 18–24 h. Then, cells were treated with medium containing 10% patient serum or EPS-conditioned medium (DMEM with 2% FCS Superior and 10% human serum from healthy individuals) for 48 or 96 h at 37°C; 18 h before lysis, cells received 10 μM 5-bromo-2′-deoxyuridine (BrdU), and BrdU incorporation was measured using the Cell Proliferation ELISA Kit (Roche Applied Science, Penzberg, Germany) according to manufacturer's protocol. For vitality assays, cells were trypsinized and total cell numbers and numbers of apoptotic/necrotic cells were determined using Trypan blue. Treatment-induced DNA fragmentation was measured by Cell Death Detection ELISA^{PLUS} (Roche Applied Science) following manufacturer's instructions.

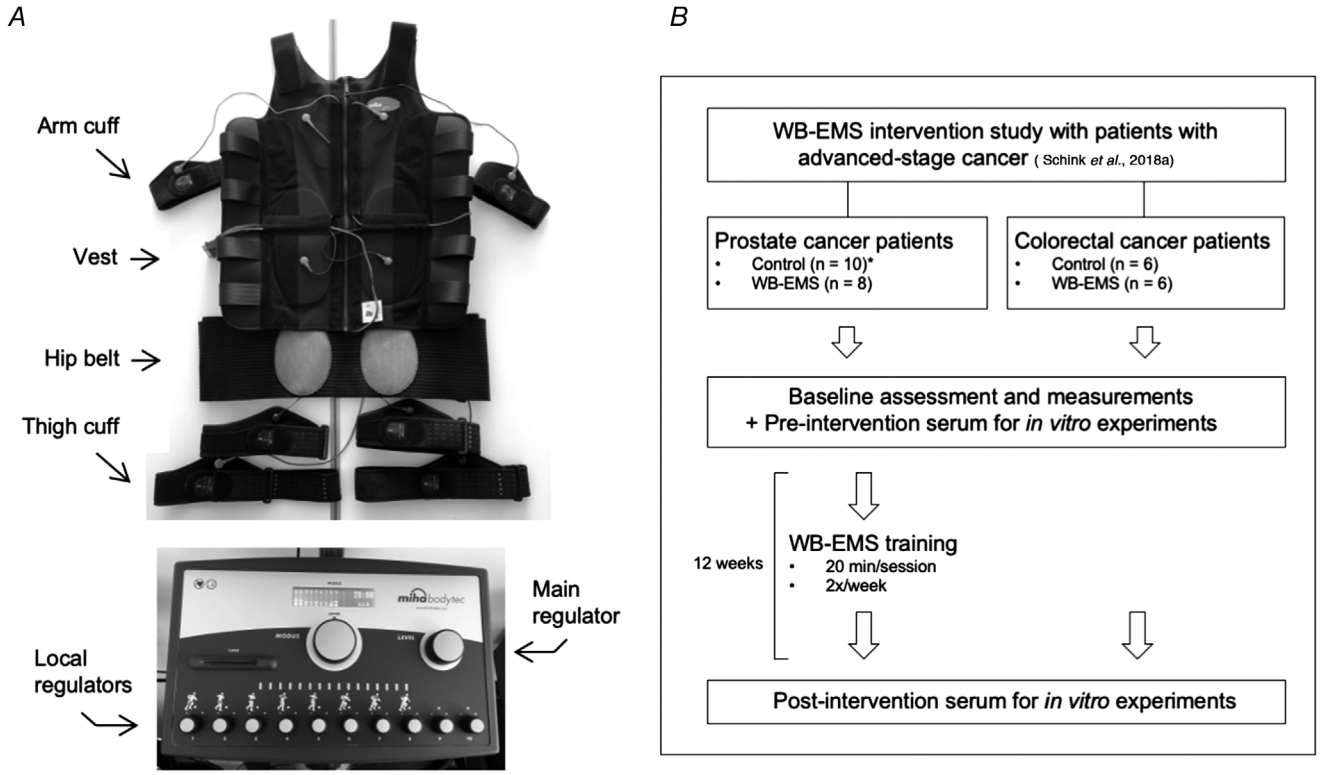


Figure 1. Whole-body electomyostimulation (WB-EMS) equipment and study flowchart
A, the upper image displays the vest, hip belt and upper-arm and -thigh cuffs with integrated electrodes. Electric stimulation was mediated by bipolar impulses (frequency 85 Hz, pulse width 350 μs; 6 s impulse phase followed by 4 s resting phase; 20 min per training session). Eight muscle groups are simultaneously addressed by the WB-EMS application (upper arms, chest, upper back, latissimus, abdomen, lower back, buttocks and thighs). The lower image shows the operating unit of the miha bodytec WB-EMS training device, with the main regulator and the local regulators for each muscle group. *B*, study overview. The patients participated in our previous controlled pilot trial (Schink *et al*. 2018*a*). *Six out of 10 control prostate cancer patients were recruited after the clinical trial from the outpatient uro-oncological unit, Medical Department 5, University Clinic Erlangen.

Table 1. Pre-intervention characteristics of the prostate and colorectal cancer patient cohorts

	Prostate cancer			Colorectal cancer		
Characteristic	Control ($n = 10$)	WB-EMS ($n = 8$)	P	Control ($n = 6$)	WB-EMS ($n = 6$)	P
Sex			—			—
Male, *n* (%)	10 (100)	8 (100)		2 (33.3)	3 (50)	
Female, *n* (%)				4 (66.7)	3 (50)	
Age (y)	68.2 ± 6.4	69.9 ± 13.3	0.729∗	53.8 ± 15.6	60.0 ± 14.6	0.496∗
Tumor stage (UICC), *n* (%)			—			—
III	—	1 (12.5)		—	1 (16.7)	
IV	10 (100)	7 (87.5)		6 (100)	5 (83.3)	
Oncological therapy, *n* (%)			—			—
CT, *n* (%)	2 (20)	—		4 (66.7)	4 (66.7)	
HT, *n* (%)	—	2 (25)		—	—	
CT + HT, *n* (%)	8 (80)	2 (25)		—	—	
Other combined therapies, *n* (%)	—	4 (50)		2 (33.3)	2 (33.3)	
Karnofsky index (%)	80 ± 7.1 ($n = 4$)	76.3 ± 13.0	0.388∗∗	75.0 ± 5.5	73.3 ± 8.2	1.0∗∗
6 min-walking distance (m)	486.3 ± 64.2 ($n = 4$)	494.4 ± 106.2	0.966∗∗	521.2 ± 112.3	520.8 ± 126.3	1.0∗∗
Body parameters						
Body weight (kg)	86.3 ± 9.4	86.9 ± 12.2	0.913∗	78.7 ± 15.9	73.6 ± 21.2	0.653∗
Weight loss in the last 3–6 month (%)	4.2 ± 5.6 ($n = 4$)	3.7 ± 5.3	0.909∗∗	8.9 ± 8.6	1.8 ± 2.8	0.058∗∗
Body mass index (kg m^{-2})	28.5 ± 2.1	27.5 ± 3.2	0.442∗	28.5 ± 6.2	25.1 ± 5.3	0.334∗
Skeletal muscle mass (kg)	26.3 ± 4.4 ($n = 4$)	28.4 ± 5.4 ($n = 7$)	0.523∗	23.1 ± 5.4	22.3 ± 7.1	0.829∗
Blood parameters						
Albumin (g l^{-1})	38.3 ± 3.8 ($n = 9$)	42.2 ± 2.6	0.034∗	40.3 ± 1.6	40.6 ± 4.0	0.867∗
C-reactive protein (mg l^{-1})	20.7 ± 23.6 ($n = 9$)	16.0 ± 35.9	0.863∗	17.9 ± 31.0	14.0 ± 28.9	0.826∗
Creatinine (mg dl^{-1})	1.0 ± 0.3 ($n = 9$)	0.9 ± 0.2	0.678∗	0.74 ± 0.1	0.8 ± 0.2	0.374∗
Haematocrit (%)	34.3 ± 4.4	38.2 ± 6.5	0.152∗	34.1 ± 3.0	38.1 ± 3.8	0.074∗
Haemoglobin (g dl^{-1})	11.4 ± 1.5	12.7 ± 2.1	0.133∗	11.7 ± 1.2	12.7 ± 1.4	0.218∗
Leucocytes (x10^3 μl^{-1})	7.6 ± 2.9	4.6 ± 2.1	0.034∗∗	5.9 ± 1.1	5.5 ± 0.8	0.853∗∗
Erythrocytes (x10^6 μl^{-1})	3.7 ± 0.4	4.2 ± 0.7	0.034∗∗	4.4 ± 0.3	4.2 ± 0.4	0.818∗∗
Thrombocytes (x10^3 μl^{-1})	248 ± 84.9	193.3 ± 56.2	0.274∗∗	276.2 ± 106.5	242.3 ± 53.3	0.513∗∗

Values are given as means (SD). Where appropriate, statistical analysis was performed using independent samples *t* test∗ or Mann–Whitney test∗∗. CT, chemotherapy; HT, hormone therapy.

Electric pulse stimulation

Per well, 1.5×10^5 primary HSMMs were seeded in six-well plates. After reaching 70–80% confluency, HSMMs were incubated for 3–4 d with DMEM–Ham F12 (Thermo Fisher Scientific) supplemented with 2% Hyclone™ horse serum (GE Healthcare, Chicago, IL, USA) at 37°C for differentiation into myotubes. Effective myogenic differentiation was checked by myogenin expression and the grade of myoblast fusion (data not shown). Medium was changed to 2 ml DMEM with 2% FCS and EPS (C-Pace EP, Ion Optix, Westwood, MA, US) was performed twice in a period of 24 h in line with the settings of a WB-EMS training unit in patients (20 min; bouts of 6 s stimulation, 4 s pause; 85 Hz pulse frequency, 400 µs pulse width, 15 V amplitude). Medium was collected 1 h after the second EPS treatment and stored at −80°C. EPS efficiency was investigated via quantitative RT-PCR (*CXCL5*, *PPARGC1A* and *MSTN* mRNA expression).

Multiplex gene expression array

For cancer gene expression analysis, 1×10^5/well low-passage LNCaP cells were seeded into 12-well plates, serum-depleted for 24 h and treated with either 10% of week 0 and week 12 serum pools from WB-EMS or control patients, or with a medium pool from EPS-treated human myotubes (from 3 independent treatments) at 37°C. The stimulation period was 24 and 72 h. Total RNA was isolated with the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. Gene expression analysis was performed using the nCounter® PanCancer Pathways Panel (730 genes from cancer-associated pathways; NanoString Technologies, Seattle, WA, USA); 100 ng total RNA from each sample was used to run the analysis with the nCounter® FLEX Analysis System (NanoString Technologies). The raw data are available in the GEO database at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE135541,

reference number GSE135541. Normalized data from treated LNCaP cells (EPS-conditioned myotube medium/unconditioned myotube medium; WB-EMS group post-intervention serum/pre-intervention serum, and control group post-intervention serum/pre-intervention serum) were compared, and potentially regulated genes were selected for further analysis. The following selection criteria were chosen: a difference in gene expression ratio of >20% in both, EPS medium-treated *vs.* untreated samples, and post- *vs.* pre-intervention samples from the WB-EMS group. Additionally, gene expression in the LNCaP cells stimulated with control cancer patient serum pools should be unaffected or inversely regulated compared to the WB-EMS ratio. Gene expression profiles were examined with the nSolver 4.0 software (NanoString Technologies). Experiments were repeated 3 times for data validation. Total RNA was isolated and reverse-transcribed cDNA was subjected to quantitative RT-PCR. The reference genes glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) and/or ribosomal protein lateral stalk subunit P0 (*RPLP0*) were used to calculate relative mRNA levels with the $2^{-\Delta\Delta C_T}$ method.

Quantitative RT-PCR

Total RNA was isolated from cells using QIAzol® reagent (Qiagen) according to manufacturer's protocol, and 0.5–1 µg was transcribed into cDNA using iScript™ synthesis kit (Bio-Rad, Hercules, CA, USA). Quantitative RT-PCR (qRT-PCR) was performed using the CFX Connect Real-Time System and CFX Manager software (Bio-Rad).

Primer sequences

Oligonucleotide synthesis was done by Metabion International AG (Planegg, Germany). For gene expression analysis of EPS efficiency and myokines, the following primers were designed/used: Human *HPRT1* (forward, f) 5′-CCTGGCGTCGTGATTAGTGAT-3′ and (reverse, r) 5′-AGACGTTCAGTCCTGTCCATAA-3′ (PrimerBank ID 164518913c1; PrimerBank Massachusetts General Hospital/The Centre for Computational and Integrative Biology/Harvard Medical School); human *CXCL5* (f) 5′-CCACTATGAGCCTCCTGTCC-3′ and (r) 5′-GATGAACTCCTTGCGTGGTC-3′; human *MSTN* (f) 5′-GAGACCCGTCGAGACTCCTAC-3′ and (r) 5′-AGTGCCTGGGTTCATGTCAAG-3′; human *PPARGC1A* (f) 5′-TGAAGACGGATTGCCCTCATT-3′ and (r) 5′-GCTGGTGCCAGTAAGAGCTT-3′ (PrimerBank ID 116284374c3); human *CCL2* (QT00212730, Qiagen); human *IGFBP3* (f) 5′-CCCTGCCGTAGAGAAATGGA-3′ and (r) 5′-CCTTCCCCTTGGTGGTGTAG-3′; human *IL5* (f) 5′-GGGAATAGGCACACTGGAGA-3′ and (r) 5′-TGCAGGTAGTCTAGGAATTGGT-3′; human *IL6* (f) 5′-AGCTCTATCTCCCCTCCAGG-3′ and (r) 5′-GAGGTGAGTGGCTGTCTGTG-3′; human *IL7* (f) 5′-CAACACAGACTCGGCAACTC-3′ and (r) 5′-GAACAAGGATCAGGGGAGGA-3′; human *IL8* (f) 5′-GCAGAGGGTTGTGGAGAAGT-3′ and (r) 5′-ACCCTACAACAGACCCACAC-3′; human *IL15* (QT00052066, Qiagen); human *VEGFA* (f) 5′-ATCCAATCGAGACCCTGGTG-3′ and (r) 5′-ATCTCTCCTATGTGCTGGCC-3′; human *BDNF* (f) 5′-GGCTTGACATCATTGGCTGAC-3′ and (r) 5′-CATTGGGCCGAACTTTCTGGT-3′. For gene array validation experiments, the following primers were designed/used: Human *RPLP0* (f) 5′-TACACCTTCCCACTTGCTGA-3′ and (r) 5′-ATCCCATATCCTCGTCCGAC-3′; human *GAPDH* (f) 5′-AACGGATTTGGTCGTATTGGG-3′ and (r) 5′-TGGAAGATGGTGATGGGATTT-3′; human *BID* (f) 5′-ACCAGAACCTACGCACCTAC-3′ and (r) 5′-CTAGGAACGCTGTTGACATGC-3′; human *CASP3* (f) 5′-ACTGGACTGTGGCATTGAGA-3′ and (r) 5′-GCACAAAGCGACTGGATGAA-3′; human *CASP7* (f) 5′-AGGGACCGAGCTTGATGATG-3′ and (r) 5′-GCACAAACCAGGAGCCTCTT-3′; human *CCNE1* (f) 5′-CAAACTCAACGTGCAAGCCT-3′ and (r) 5′-GCAGAAGAGGGTGTTGCTCA-3′; human *CDKN1B* (f) 5′-TCTGAGGACACGCATTTGGT-3′ and (r) 5′-CAGAACCGGCATTTGGGGAA-3′; human *DUSP8* (f) 5′-TACCCATGAGCCTCTCCCA-3′ and (r) 5′-CAGGAGTTGCTGGCGTTG-3′; human *GHR* (f) 5′-CCCAGTTCCAGTTCCAAAGATT-3′ and (r) 5′-TCCTCAGTCTTTTCATCTGGCT-3′; human *WNT10A* (f) 5′-GGCAACCCGTCAGTCTGTCT-3′ and (r) 5′-CATTCCCCACCTCCCATCT-3′ (Nakamura *et al.* 2005).

Cytokine array

For simultaneous detection of cytokines/myokines in the medium from untreated or EPS-treated primary human myotubes, a Human Cytokine Antibody Array (120 targets; Abcam, Cambridge, UK) was performed. The assay was done with a pool of myotube medium from three independent EPS approaches according to the manufacturer's protocol. For chemiluminescence detection the Amersham 600 Imager (GE Healthcare, Pittsburgh, PA, USA) was used. Signals were analysed using ImageJ software (National Institute of Health, Bethesda, MD,USA; RRID:SCR_003070). Normalization of the raw densitometry data was performed according to manufacturer's recommendations. After subtracting the mean density of the negative controls (background), the density of each cytokine spot was normalized to the mean positive control signal (for each membrane). The values of the untreated and the EPS-treated medium pools

were compared and already established myokines with an upregulation of >10% in the EPS pool were selected.

Immunoblotting

Per well, 8×10^4 LNCaP cells were seeded into 12-well plates, and after overnight incubation at 37°C, cells were serum-starved (0.1% FCS) for 18 h. Cells were treated with medium containing 10% prostate cancer patient serum or EPS-conditioned myotube medium for 96 h at 37°C. Cells were lysed in cell lysis buffer (0.5% NP-40, 150 mM NaCl, 20 mM Tris pH 7.4, 10 mM phenylmethylsulfonyl fluoride, phosphatase inhibitors (PhosSTOP, Roche Diagnostics), protease inhibitors (Protease Inhibitor Cocktail Set I, Calbiochem, Merck KGaA). Cell extracts were fractionated by SDS-PAGE and analysed by immunoblotting with antibodies specific for poly (ADP-ribose) polymerase (PARP; recognizes full length, 116 kDa, and large fragment, 89 kDa) and cleaved PARP (Asp214; large fragment, 89 kDa; both from Cell Signaling Technology, Danvers, MA, USA; RRID:AB_2 160 739, RRID:AB_10 699 459). Using horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and enhanced chemiluminescence, the membranes were analysed with the Amersham 600 Imager and the signals were quantified using ImageJ software.

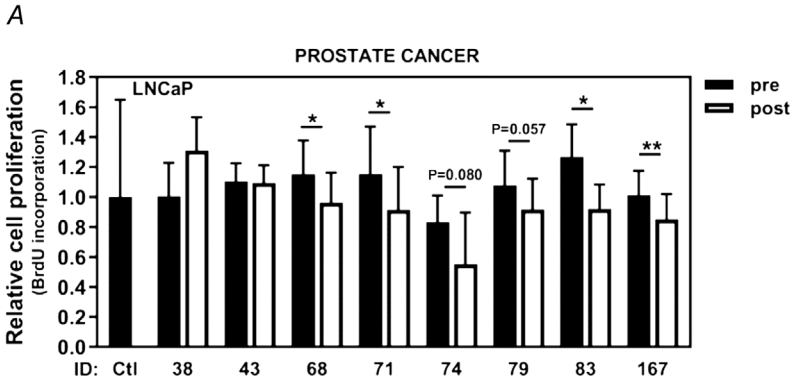
Statistics

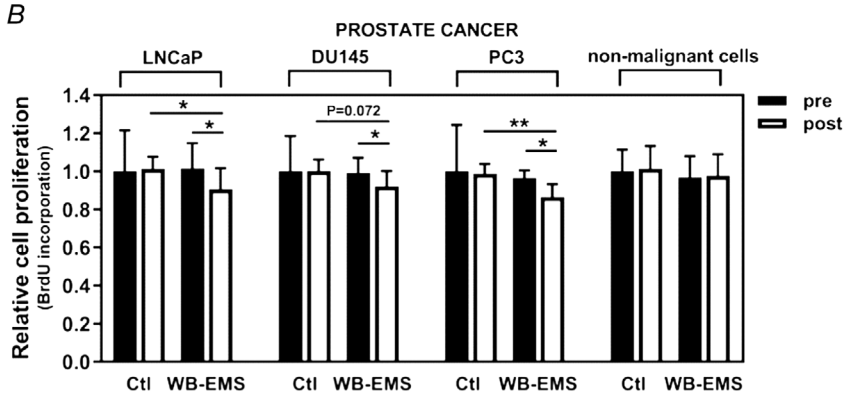
Study group differences in Table 1 were analysed by independent samples Student's *t* test or Mann–Whitney test. Graphs depict the mean (SD). The respective number of experiments is stated in the figure legends. For statistical analysis of BrdU incorporation and DNA fragmentation after stimulation with patient serum, a paired (post- *vs.* pre-intervention data within a patient group) or unpaired two-tailed Student's *t* test (post-intervention data between the patient groups) was applied. For analysis of cell counting and Trypan blue, the Wilcoxon signed-rank (post- *vs.* pre-intervention values within a patient group) and the Mann–Whitney test (post-intervention data between the patient groups) were used. For data evaluation of BrdU incorporation, DNA fragmentation and PARP cleavage (Immunoblot) after stimulation with EPS-conditioned myotube medium, an unpaired two-tailed Student's *t* test was applied, and for cell counting data analysis after EPS-medium treatment, the Mann-Whitney test was used. For statistics on gene expression data, we applied a paired (post- *vs.* pre-intervention WB-EMS serum) or unpaired (post-intervention serum between the patient groups; EPS-conditioned *vs.* unconditioned medium) two-tailed Student's *t* test. EPS marker and myokine expression in human myotubes after EPS application was examined using an unpaired two-tailed Student's *t* test. Statistical analysis was performed using GraphPad Prism 8.1.2 (GraphPad Software, San Diego, CA, USA; RRID:SCR_0 02798). *P* values of < 0.05 were considered statistically significant.

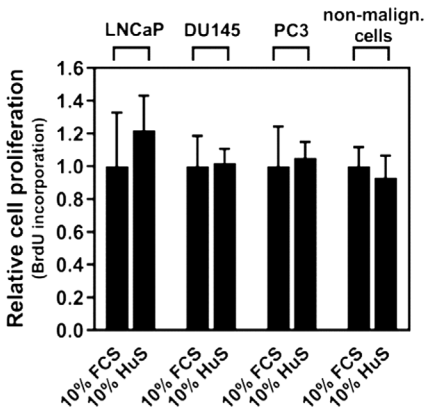
Results and discussion

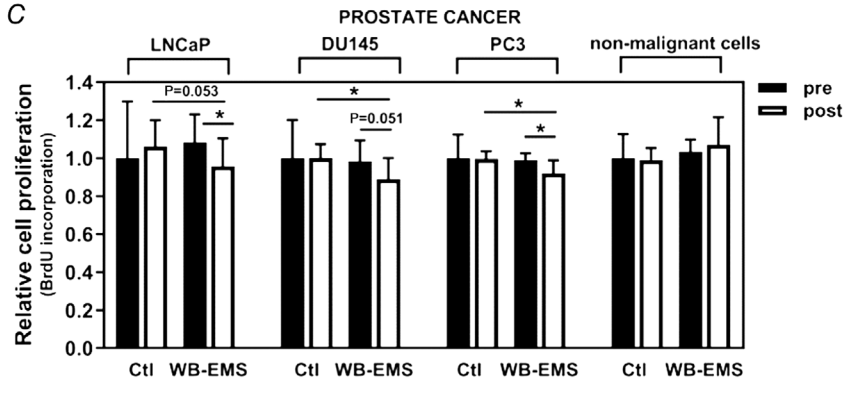
In the initial clinical trial, advanced-stage cancer patients were examined regarding the effect of WB-EMS (for 12 weeks, 2x/week, 20 min/session; Fig. 1) on body weight, skeletal muscle mass and physical function (Schink *et al.* 2018*a*). The study flowchart for our *in vitro* investigation focusing on prostate and colorectal cancer patients is shown in Fig. 1*B*. Table 1 displays the baseline demographic and disease characteristics of the included patients. No significant difference between the study groups within each cancer type were observed regarding performance status (Karnofsky index) and physical functioning (6 min-walk test) as well as in body parameters. A relative weight loss of >5% of the body weight over 3–6 month prior to study entry indicated that some participants may have had cancer cachexia (prostate cancer cohort: 20% of the controls, 25% of the WB-EMS patients; colorectal cancer: 66.7% of the controls, 16.7% of the WB-EMS patients). The blood parameter measurement in the prostate cancer cohort showed significant differences between control and WB-EMS patients regarding albumin levels, leucocyte and erythrocyte count.

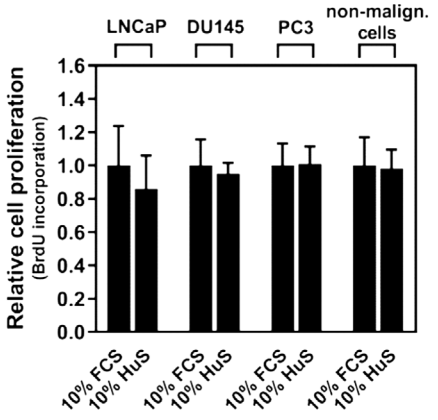
Our research aim was to use the patient serum collected before the intervention at study entry and post-intervention in order to analyse the anti-cancer effects of WB-EMS exercise on cancer cells *in vitro*. We stimulated the human androgen-sensitive prostate cancer cell line LNCaP with pre- and post-intervention serum from advanced prostate cancer patients with ($n = 8$) or without WB-EMS training ($n = 10$) for 96 h. For the first time, we could show that cancer cell growth, determined by proliferation assay and cell counting, was inhibited with WB-EMS exercise serum (Fig. 2*A*). Six out of eight patient sera clearly triggered a lower cell proliferation rate (ID 38: $P = 0.061$; 43: $P = 0.723$; 68: $P = 0.024$; 71: $P = 0.019$; 74: $P = 0.080$; 79: $P = 0.057$; 83: $P = 0.014$; 167: $P = 0.009$). So far, we have no definite explanation of why the serum from two WB-EMS patients was without effect. We can speculate that differences in the cancer therapy regimen or individual factors like other medication might be responsible. The BrdU uptake after incubation with the pre-intervention serum from WB-EMS patients did not significantly differ from the values obtained after stimulation with a pool of pre-intervention control patient serum ($n = 10$; Fig. 2*A*, left single bar). By that, we could exclude that the growth

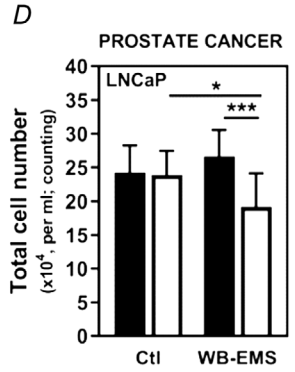


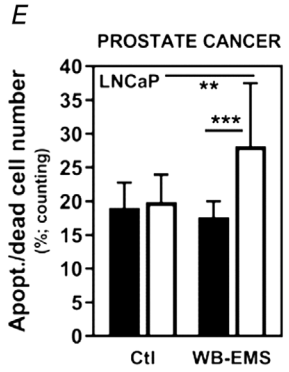












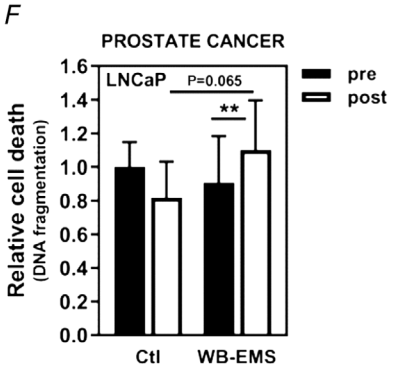


Figure 2. Prostate cancer patient serum after WB-EMS inhibits prostate carcinoma cell growth
A, the prostate cancer cell line LNCaP was serum-starved in RPMI medium containing 0.1% FCS for 18–24 h at 37°C, and stimulated for 96 h with 10% serum from stage III/IV prostate cancer patients taken at study entry (pre) or after a 12-week (post) WB-EMS exercise regimen. Cell proliferation was measured via BrdU incorporation into DNA and is shown relative to the values after stimulation with a pre-intervention serum pool from control patients ($n = 10$). Means (SD) of at least 6 independent experiments per patient are shown. *B*, prostate cancer cells (LNCaP, DU145, PC3) and the control non-malignant cells HEK293T were serum-starved (RPMI with 0.1% FCS or DMEM with 0.1% FCS, respectively) and incubated for 96 h at 37°C with serum pools from WB-EMS ($n = 8$) or control patients ($n = 10$) with advanced prostate tumours, and BrdU uptake was measured (left). Statistical analysis on the proliferation data with post-intervention serum between the two patient groups revealed a distinct difference in the growth response upon the post-WB-EMS serum pool compared to the control pool (LNCaP, $P = 0.035$; DU145, $P = 0.073$; PC3, $P = 0.002$). Means (SD) of at least 7 independent experiments per cell line are shown. The right graph depicts the growth response of the cells upon 10% FCS compared to 10% human serum (pre-intervention control pool from prostate cancer patients; mean (SD), $n = 5$–8). *C*, similar to Fig. 2*B*, cells were serum-starved but incubated for a shorter period (48 h) with control and WB-EMS serum pools at 37°C, and BrdU incorporation was measured (left). Statistical analysis of the proliferation upon incubation with post-intervention serum between the groups showed also a significant difference (LNCaP $P = 0.053$; DU145 $P = 0.023$; PC3 $= 0.044$). Means (SD) of at least 5 independent experiments per cell line are shown. The right graph depicts the cell proliferation upon 10% human compared to 10% bovine serum (mean (SD), $n = 5$–7). *D* and *E*, LNCaP were serum-starved and treated with serum pools from WB-EMS or control patients at 37°C. After 96 h, cell viability was examined by determination of total cell numbers (*D*; numbers per ml $\times 10^4$) and the number of apoptotic/dead cells via Trypan blue inclusion (*E*; percentage of total cell number; statistical analysis between the groups after post-intervention serum incubation, $P = 0.003$). Means (SD) of 11 independent experiments. *F*, apoptotic DNA fragmentation was measured after 48 h of WB-EMS serum stimulation (shown relative to pre-intervention control serum values; statistical analysis between the groups after post-intervention serum incubation, $P = 0.065$). Means (SD) of 8 independent experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; paired and unpaired two-tailed Student's *t* test in *A*, *B* and *C*; Wilcoxon signed-rank and Mann–Whitney test in *D*, *E* and *F*.

inhibitory effect seen with post- *vs.* pre-intervention serum in the WB-EMS patients is exclusively caused by an already at baseline existing significant difference in the cell proliferative behaviour, compared to control serum. Yet, a subtle higher proliferation rate induced by some pre-intervention WB-EMS patient sera might contribute to the anti-proliferative effects shown in Fig. 2*A*. Since androgens affect the growth of hormone-sensitive prostate cancer cells, we measured the testosterone amount in each prostate cancer patient serum (Table 2). Of note, 80% of the control patients and all WB-EMS patients were on anti-androgen therapy throughout the trial phase (Table 1). In post-intervention testosterone levels, 66.7% of the tested control as well as WB-EMS patient sera showed a decrease. However, the comparison of the testosterone measurements with the BrdU uptake data in Fig. 2*A* revealed that reduced LNCaP proliferation upon post-WB-EMS serum is not necessarily caused by a lower testosterone amount (e.g. patients 79 and 167). On the other hand, patient 74 had a high pre-intervention testosterone level (3.17 ng ml^{-1}), which dropped to 0.24 ng ml^{-1} post-intervention; patient 74's serum displayed the most pronounced LNCaP cell growth inhibition (−33.7%; Fig. 2*A*). In order to investigate if the reduction in serum testosterone is responsible for this, we stimulated the hormone-insensitive prostate cancer cell

Table 2. Serum testosterone measurements of the prostate cancer cohort. Values are given as mean from duplicate measurements

Control ($n = 6$)			WB-EMS ($n = 6$)		
	Serum testosterone (ng ml^{-1})			Serum testosterone (ng ml^{-1})	
ID	Pre	Post	ID	Pre	Post
K01	0.17	0.12	68	0.50	0.47
K02	0.12	0.33	71	0.38	0.12
K04	0.08	0.06	74	3.17	0.24
K05	0.12	0.03	79	0.08	0.08
K07	0.24	0.27	83	0.23	0.20
K08	0.52	0.32	167	0.15	0.32
Mean ± SD	0.21 ± 0.22	0.14 ± 0.13	Mean ± SD	0.75 ± 1.20	0.24 ± 0.14
P	0.080			0.339	

Pre- and post-intervention serum from 6 patients per group could be examined. No significant difference in testosterone levels (ng ml^{-1}) between pre- and post-intervention serum within each group was determined (mean (SD); two-tailed paired Student's *t* test).

lines DU145 and PC3 with pre- and post-intervention serum from patient 74. We also observed a decreased cell proliferation in these hormone-insensitive cells upon post-WB-EMS serum, but not as distinct (data not shown).

A post-intervention serum pool from all WB-EMS patients ($n = 8$) attenuated human prostate cancer cell proliferation after 96 h (Fig. 2*B*, left; LNCaP, −10.9%, $P = 0.014$; DU145, −7.0%, $P = 0.048$; PC3, −10.4%, $P = 0.011$), in contrast to serum taken prior to exercise and from control patients. Remarkably, the growth of human non-malignant HEK293T cells was not inhibited by WB-EMS serum (Fig. 2*B*, left; for additional statistical analysis, refer to the legend of Fig. 2). The mean serum testosterone levels shown in Table 2 reflect the testosterone level in the patient serum pools. The testosterone amount in the post-intervention serum from both groups was lower compared to pre-intervention samples (control: 0.21 ± 0.22 ng ml^{-1} (pre) to 0.14 ± 0.13 ng ml^{-1} (post), $P = 0.080$; WB-EMS: 0.75 ± 1.20 ng ml^{-1} (pre) to 0.24 ± 0.14 ng ml^{-1} (post), $P = 0.339$; Table 2), indicating again that changes in serum testosterone do not play a significant role in our cancer cell growth experiments. Of note, we checked whether human serum is an adequate growth environment for the prostate cancer cells. The BrdU incorporation rate after stimulation with 10% baseline serum pools compared to 10% FCS (Fig. 2*B*, right graph) revealed no significant difference in the proliferation behaviour. Patient serum incubation on prostate cancer and non-cancer cells for a shorter period (48 h) showed similar growth-inhibitory effects of the post-WB-EMS serum on the cancer cells (Fig. 2*C*, left; LNCaP, −11.6%, $P = 0.018$; DU145, −9.6%, $P = 0.051$; PC3, −7.0%, $P = 0.042$). Again, the growth response of the cell lines did not significantly differ under treatment with human compared to bovine serum (Fig. 2*C*, right). Taken together, these data strongly suggest cancer cell-specific effects of exercise-mediated mechanisms that attenuate cell proliferation.

We also observed that LNCaP viability was impaired by the WB-EMS-conditioned patient serum pool (Fig. 2*D*–*F*). Total cell count was significantly reduced (−27.9%, $P = 0.001$; Fig. 2*D*). Statistical analysis between the groups indicated that the cell numbers after incubation with post-WB-EMS serum are also significantly lower ($P = 0.022$) compared to control serum. Furthermore, apoptotic events, measured by apoptotic/dead cell numbers (from 17.6% to 28.1%, $P = 0.001$; Fig. 2*E*) and DNA fragmentation (+21.2%, $P = 0.035$; Fig. 2*F*), were increased upon post-WB-EMS serum. Experimental variation, e.g. incubation time, might be responsible for the difference in apoptotic response upon incubation with post- compared to pre-intervention control serum between the two assays (Fig. 2*E* und *F*).

Similar to these data in prostate cancer cells, human colon carcinoma cells were also affected after treatment with a serum pool from patients with advanced colorectal cancer after WB-EMS. Upon incubation with post-exercise serum for 96 or 48 h, proliferation was decreased by 9.1% ($P = 0.031$; Fig. 3*A*, left) or by 13.2% ($P = 0.021$; Fig. 3*B*, left), respectively, in the human colon cancer cell line HT29 cells; proliferation of the non-malignant HEK293T cells was uninhibited by post-WB-EMS serum. We also checked HT29 cell proliferation after stimulation with 10% human serum compared to 10% FCS and detected no significant difference in BrdU uptake (Fig. 3*A* and *B*, right graphs). As seen in prostate cancer cells, HT29 total cell number was diminished upon exercise serum (−25.8%, $P = 0.008$; Fig. 3*C*). Concomitantly, apoptosis/necrosis was increased as detected by Trypan blue inclusion (from 9.4% to 14.4%, $P = 0.047$; Fig. 3*D*).

Taken together, these results imply that WB-EMS induces defensive humoral factors in cancer patients that target malignant cell growth and viability, independent of their hormonal sensitivity, while non-tumour cell growth is unaffected, a hitherto very important finding. Interestingly, several *in vitro* and *in vivo* studies focusing on prostate cancer described that serum from healthy individuals after acute and long-term exercise contains lower amounts of epidermal growth factor (EGF), fibroblast growth factor 4 (FGF-4) and insulin-like growth factor 1 (IGF-1) as well as an increased level of insulin-like growth factor-binding protein 1 (IGFBP-1) (Ngo *et al.* 2002; Rundqvist *et al.* 2013). It is possible that deregulated protein levels might also be responsible, at least in part, for the inhibitory effects of WB-EMS on prostate cancer cell growth seen in our experiments. Barnard and colleagues showed that an exercise-mediated increase in p53 and p21 protein level and a concomitant decrease in the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) can be reasons for reduced prostate carcinoma cell growth (Barnard *et al.* 2007). Furthermore, serum from early-stage prostate cancer patients that underwent extensive lifestyle changes including unmonitored regular exercise suppressed prostate cancer cell growth (Ornish *et al.* 2005).

EPS of cultured skeletal muscle cells at the myotube stage can be used as an *in vitro* model of exercise (Nikolic *et al.* 2012, 2017; Scheler *et al.* 2013). We applied EPS to human myotubes with parameters similar to the WB-EMS training (85 Hz, 15 V, 400 µs band width, 6 s stimulation, 4 s rest; 2 × 20 min on two consecutive days), and could show that known EPS-responsive genes like C-X-C motif chemokine 5 (*CXCL5*; $P = 0.0004$) and peroxisome proliferator-activated receptor γ coactivator 1α (*PPARGC1A*; $P = 0.003$) are increased, and myostatin (*MSTN*) is downregulated ($P = 0.072$; (Fig. 4*A*) (Nedachi *et al.* 2008; Nikolic *et al.* 2012; Tarum *et al.* 2017). In accordance with the observed WB-EMS serum

effects, we found that EPS-conditioned medium decreased proliferation of LNCaP cells by 7.7% ($P = 0.007$), of PC3 cells by 7.4% ($P = 0.0003$) and with lower efficacy in DU145 cells (−3.5%, $P = 0.003$; Fig. 4*B*). In line with that, total LNCaP cell count was significantly reduced after EPS medium incubation (−18.8%, $P = 0.005$, left bars), while cell death, quantified by apoptotic/dead cell numbers (from 14.3% to 19.8%, $P = 0.0007$, right bars; Fig. 4*C*) and DNA fragmentation (+ 83%, $P = 0.017$; Fig. 4*D*), was increased. We obtained similar results in colon cancer cells. EPS-conditioned medium reduced proliferation of HT29 cells by 10.6% ($P = 0.0007$; Fig. 4*B*). Total HT29 cell count was also significantly decreased (−15.4%, $P = 0.025$, left bars), while concomitantly, the apoptotic/dead cell number (from 13.6% to 19.0%, $P = 0.014$, right bars; Fig. 4*E*) was enhanced. In contrast to that, non-malignant HEK293T cell growth was not significantly affected by the EPS medium ($P = 0.307$; Fig. 4*B*). These data indicate that

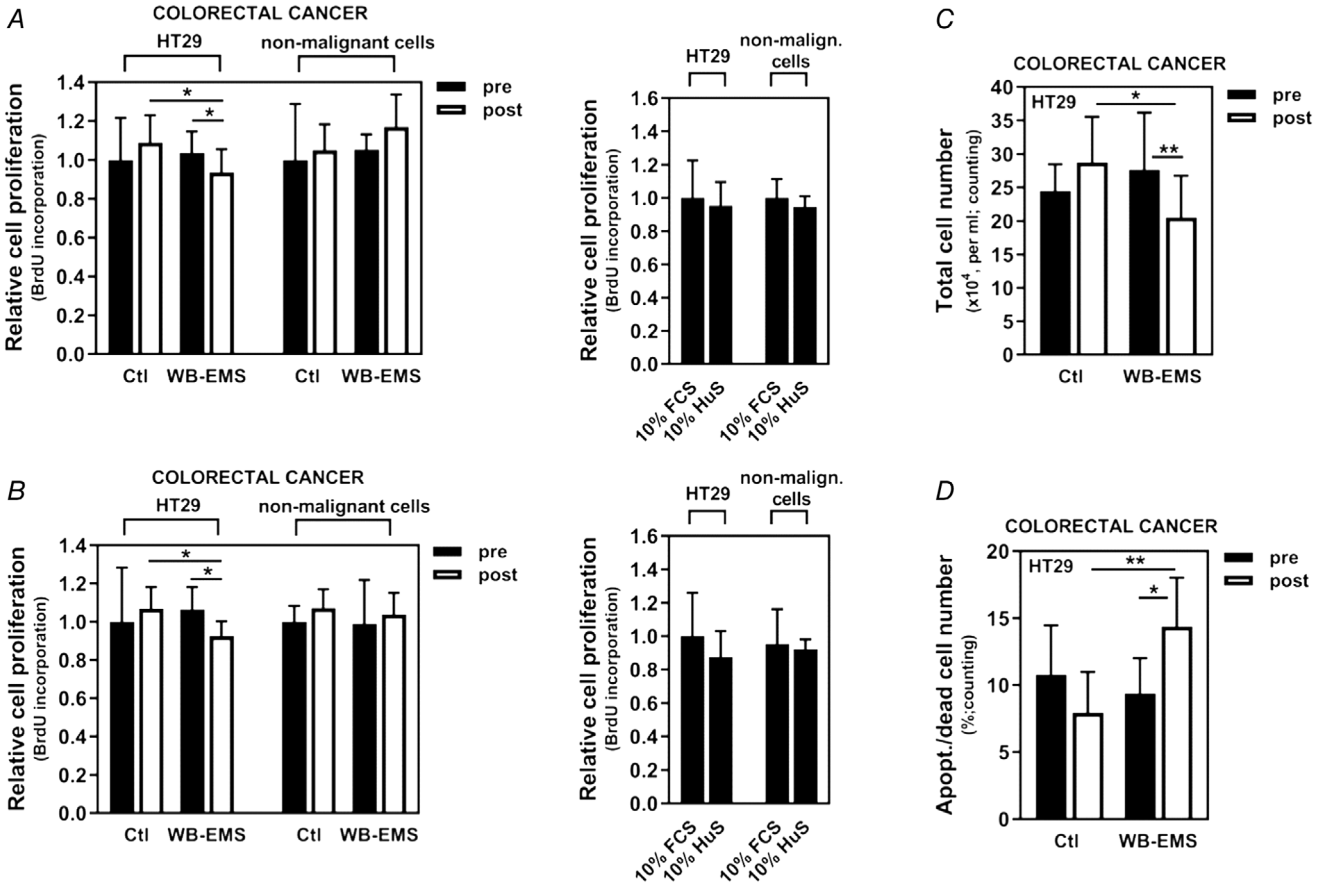


Figure 3. WB-EMS-conditioned serum from colorectal cancer patients attenuates colon carcinoma cell viability
A, HT29 colon cancer and non-malignant HEK293T cells were serum-starved (DMEM with 0.1% FCS) and incubated for 96 h at 37°C with serum pools from WB-EMS ($n = 6$) or control patients ($n = 6$) with advanced colorectal tumours. BrdU incorporation into DNA was measured via ELISA. Additional significant difference in the proliferation response was found upon incubation with post-intervention serum pools from both study groups ($P = 0.046$). Means (SD) of at least 4 independent experiments are shown. The right graph depicts cell proliferation under incubation with 10% FCS *vs*. 10% human serum (pre-intervention, colorectal cancer patients; mean (SD), $n = 3$). *B*, similar to *A*, cells were serum-starved, but incubated for a shorter period (48 h) at 37°C with control and WB-EMS serum pools, and BrdU uptake was determined. Additional significant difference in the growth response was detected by comparing the data from both study groups after treatment with post-intervention serum pools ($P = 0.013$). Means (SD) of at least 3 independent experiments are shown. The right graph depicts BrdU uptake upon incubation with 10% FCS *vs*. 10% human serum (pre-intervention, colorectal cancer patients; mean (SD), $n = 3$). *C* and *D*, serum-depleted HT29 cells were treated with WB-EMS and control serum pools; 96 h later, cell viability was determined by cell counting (*C*; numbers per ml, $\times 10^4$; statistical analysis between the groups after post-intervention serum incubation, $P = 0.039$). The number of apoptotic/dead cells was measured via Trypan blue inclusion (*D*; percentage of total cell number; statistical analysis between the groups after post-intervention serum incubation, $P = 0.001$). Means (SD) of at least 7 independent experiments. $^*P < 0.05$, $^{**}P < 0.01$; paired and unpaired two-tailed Student's *t* test in *A* and *B*; Wilcoxon signed-rank and Mann–Whitney test in *C* and *D*.

mechanisms induced by muscle cell stimulation, either *in vivo* (WB-EMS) or *in vitro* (EPS), are responsible for cancer cell growth inhibition. Moreover, the direct comparison of both approaches with yet very similar effects allow us to eliminate all non-muscle origins of respective soluble factors and track those down to muscle-secreted modulators, for which myokines are prime candidates (Karstoft & Pedersen, 2016; Hoffmann and Weigert, 2017; Piccirillo, 2019).

Stimulated skeletal muscle releases specific cytokines and growth factors which facilitate communication with various body tissues. Hundreds of secretory proteins are considered as myokines, including interleukins like IL-6 and IL-15, and growth factors like fibroblast growth factor 21 (FGF-21) and brain-derived neurotrophic factor (BDNF) (Aoi *et al.* 2013; Schnyder and Handschin, 2015; Karstoft and Pedersen, 2016; Whitham & Febbraio, 2016). We were curious about the effectiveness of our EPS approach to induce myokine expression and/or release. We analysed EPS-conditioned medium and the medium from untreated myotubes regarding their cytokine/myokine composition. A protein array revealed that several established myokine are induced and/or secreted by our EPS protocol (Table 3) (Raschke *et al.* 2013; Scheler *et al.* 2013). Together with corresponding gene expression experiments we could show that the following myokines are upregulated by EPS (Table 3): BDNF (fold change protein 2.58; fold change mRNA 1.41 ± 0.49; $P = 0.0332$), CCL-2 (1.12; 1.35 ± 0.51; $P = 0.057$), IGFBP-3 (1.13; 2.31 ± 1.01; $P = 0.001$), IL-5 (6.40; 3.18 ± 3.58; $P = 0.107$), IL-6 (1.49; 3.91 ± 1.96; $P = 0.0002$), IL-7 (protein not detectable; 3.34 ± 1.07; $P = 0.0001$), IL-8 (1.42;

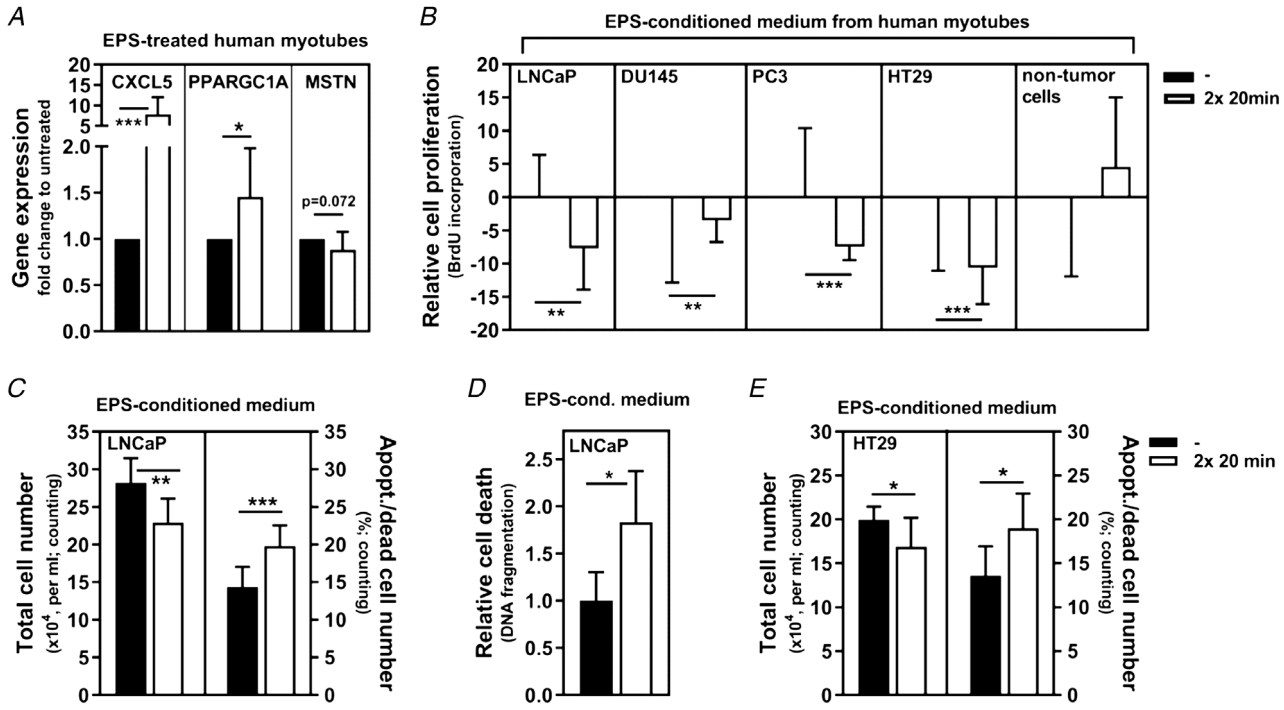


Figure 4. Prostate and colon cancer cell proliferation and apoptosis are affected by EPS-conditioned medium from human myotubes

A, human myotubes were treated with EPS (6 s stimulation followed by a 4 s pause, 85 Hz, pulse width of 400 μs, 15 V) for 20 min on 2 consecutive days. After another 1 h, total RNA was isolated and analysed for EPS marker expression (*CXCL5*, *PPARGC1A* and *MSTN*). The graph depicts the means (SD) from at least 8 representative experiments. *B*, prostate cancer (LNCaP, DU145, PC3), colon cancer (HT29) and non-cancer (HEK293T) cells were serum-starved and then treated with EPS-conditioned medium from *in vitro*-differentiated human myotubes for 48 h at 37°C. The conditioned medium contained 2% FCS and 10% human serum from 12 randomly selected healthy individuals, who were not associated with the clinical study. Cell proliferation was measured by BrdU incorporation. The graph shows the relative proliferation of untreated cells as 0%, and the percentage difference in proliferation (after treatment with EPS-conditioned compared to unconditioned medium). Means (SD) of at least 4 independent experiments. *C* and *D*, the effect of EPS-conditioned myotube medium on total (per ml $\times 10^4$) and apoptotic/dead LNCaP cell numbers was measured by cell counting and Trypan blue inclusion (*C*) and by DNA fragmentation (*D*). Means (SD) of 9–10 (*C*) and 5 (*D*) independent experiments are shown. *E*, total and apoptotic/dead HT29 cell numbers upon EPS myotube medium were analysed as in *C*. Means (SD) of 9 independent experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; unpaired two-tailed Student's *t* test in *A*, *B* and *D*; Wilcoxon signed-rank test in *C* and *E*.

Table 3. Established myokines are regulated by electric pulse stimulation based on WB-EMS training parameters

Myokine	Cytokine array (medium) EPS-conditioned/ unconditioned (fold change)	Gene expression (myotubes) EPS-treated/ untreated, mean ± SD (fold change)	*P*
BDNF	2.58	1.41 ± 0.49	0.033
CCL-2	1.12	1.35 ± 0.51	0.057
IGFBP-3	1.13	2.31 ± 1.01	0.001
IL-5	6.40	3.18 ± 3.58	0.107
IL-6	1.49	3.91 ± 1.96	0.0002
IL-7	ND	3.34 ± 1.07	0.0001
IL-8	1.42	1.73 ± 0.82	0.019
IL-15	0.78	2.06 ± 0.50	0.0001
VEGFA*	1.63	2.12 ± 0.76	0.0004

Primary human myotubes were treated with EPS (85 Hz, 400 μs pulse width, 6 s stimulation followed by 4 s rest, 15 Volt, 20 min) on two consecutive days. A myotube medium pool from three independent EPS treatments was used for a cytokine array in order to identify EPS-regulated protein factors. The fold change in myokine protein expression in myotube EPS-conditioned *vs.* unconditioned medium is shown. The same set of myokines was analysed regarding their mRNA expression in EPS-treated human myotubes. *VEGF protein was not specified for the cytokine array, but *VEGFA*-specific primers were used for gene expression analysis (fold change to untreated; mean (SD) from 7–9 independent experiments; two-tailed unpaired Student's *t* test). ND, not detectable.

1.73 ± 0.82; $P = 0.019$), and vascular endothelial growth factor (VEGF/VEGFA; 1.63; 2.12 ± 0.76; $P = 0.0004$). No increase in IL-15 protein secretion could be determined (0.78), but IL-15 mRNA level was elevated (2.06 ± 0.50, $P = 0.0001$). Other established myokines like FGF-21, leukaemia inhibitory factor and myonectin were not included in the cytokine array (Schnyder & Handschin, 2015; Karstoft and Pedersen, 2016; Whitham & Febbraio, 2016).

As mentioned before, Hojman *et al.* (2011) showed for the first time that conditioned serum from exercising mice decreases human cancer cell viability via exercise-induced factors. They attributed part of the observed pro-apoptotic effects of exercise serum to the muscle-derived factor Oncostatin M. Gannon *et al.* (2015) found that Irisin, a pivotal protein mediating several beneficial effects of exercise on the body, affects breast cancer cell viability *in vitro*. Also, exercise-induced secreted protein acidic and rich in cysteine (SPARC) was shown to suppress colon tumorigenesis *in vivo*, and decreases proliferation and concomitantly increases apoptosis of colon carcinoma cells *in vitro* (Aoi *et al.* 2013). It will be very interesting to examine if myokines play a role in WB-EMS-mediated interference with cancer cell growth.

In our study here, we were curious how cancer cell gene expression is affected by exercise, a research area involving epigenetic changes as well as modulation of up-stream signal transduction networks. We performed a gene expression screen with 730 cancer-associated genes on LNCaP prostate cancer cells stimulated with either WB-EMS or control patient serum, or with EPS-conditioned or unconditioned medium from human myotubes. After incubation for 24 and 72 h, the expression of 11 (4 up, 7 down) and 15 (6 up, 9 down) genes, respectively, was equally regulated by both treatments (Fig. 5*A*). From these hits, we chose to examine the expression of genes (potentially) involved in cancer cell proliferation and apoptosis (Table 4).

In validation experiments, we observed in the 24 h-response gene group (Fig. 5*B*) that mRNA expression of cyclin E1 (*CCNE1*) is downregulated by treatment with post-WB-EMS serum ($P = 0.004$) as well as with EPS-conditioned medium ($P = 0.1379$). Furthermore, post-intervention *CCNE1* mRNA levels significantly differed between the groups ($P = 0.014$). Cyclins are key players during cell cycle and regulate cyclin-dependent kinases. The complex of cyclin-dependent kinase 2 (CDK-2) and cyclin E is essential to drive G1/S transition (Malumbres & Barbacid, 2009). Cyclin E1overexpression seems to be associated with poor survival of cancer patients (Zhao *et al.* 2018). A study in prostate cancer cells suggested that downregulation of cyclin E1 suppresses proliferation and induces G1/S arrest (Zhang *et al.* 2019), which is in line with our results.

We also found significantly lower levels of growth hormone receptor (*GHR*) mRNA in cells treated with post-WB-EMS serum ($P = 0.041$) as well as EPS medium ($P = 0.018$). We also detected significantly different *GHR* levels when comparing mRNA expression upon post-intervention serum between the groups ($P = 0.0001$). The observed downregulation might be in part responsible for the anti-proliferative effects of exercise via WB-EMS. It was shown, for example, that *GHR* mRNA expression is 80% higher in prostate carcinomas than in benign prostate hyperplasia, and GH/GHR-mediated production of IGF-1 seemed to play an important role in tumour progression (Weiss-Messer *et al.* 2004; Dehkhoda *et al.* 2018).

Moreover, we discovered that treatment with EPS-conditioned medium clearly decreased Wnt family member 10A (*WNT10A*) mRNA expression levels ($P = 0.057$) in LNCaP cells. We also found a strong trend towards a downregulation with WB-EMS serum, and a significant difference in *WNT10A* mRNA levels between the groups upon post-intervention serum ($P = 0.027$). Wnt signalling was discovered in cancer models, and mutations in Wnt pathways are frequently observed in carcinomas. In accordance with our data, knockdown of Wnt-10A suppressed proliferation of, for example,

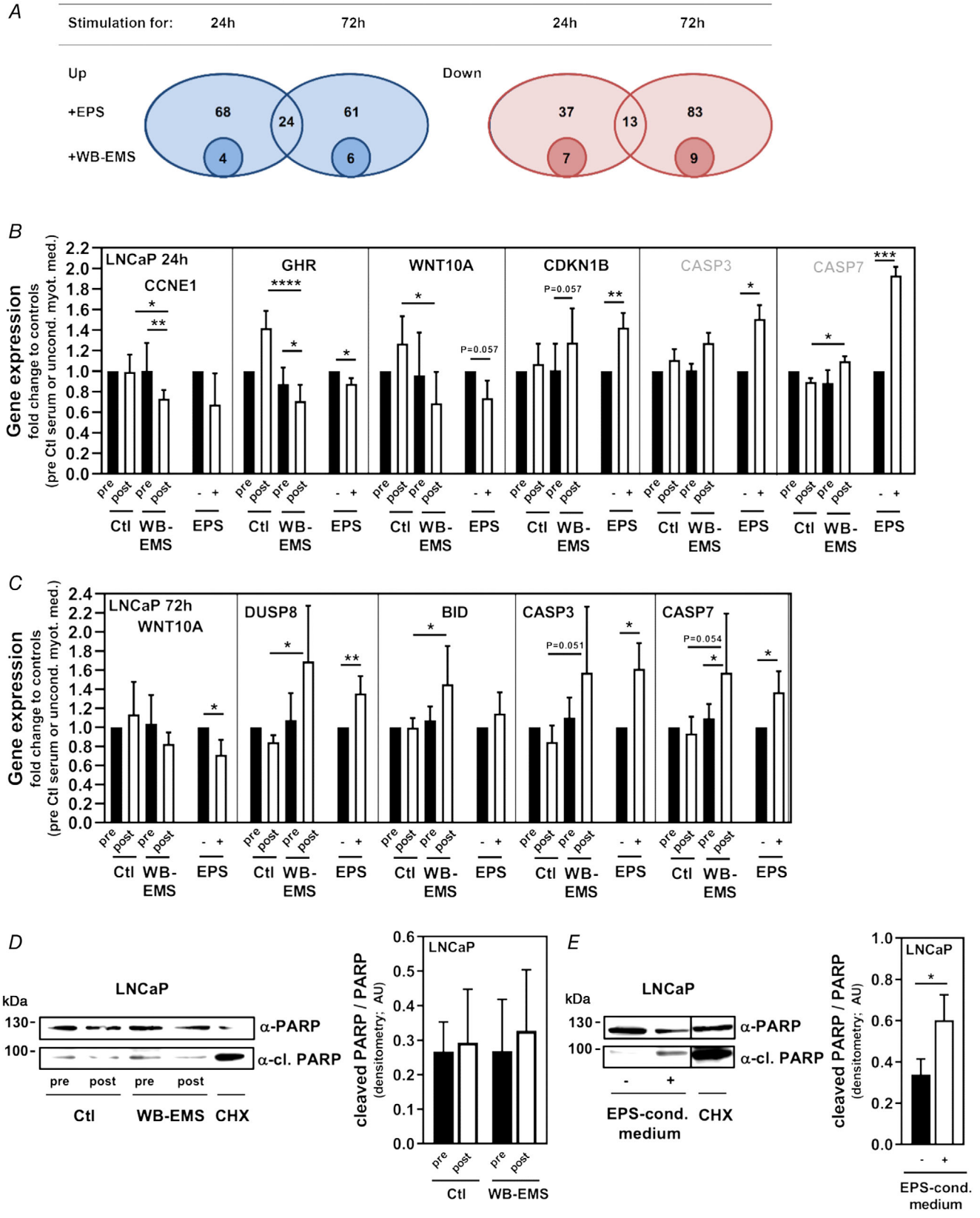


Figure 5. Specific genes associated with prostate cancer cell proliferation and apoptosis are regulated by exercise

renal and ovarian cancer cells (Hsu *et al.* 2012; Li *et al.* 2017).

Additionally, we detected that the mRNA expression of the cell cycle-dependent kinase inhibitor 1B (*CDKN1B*) is increased upon the EPS medium stimulus ($P = 0.007$) and the post-WB-EMS serum ($P = 0.057$). CDKN-1B controls the cell cycle, i.e. G0 to S phase transition, by regulating the activity of the cyclin E–CDK-2 complex (Chu *et al.* 2008). Thus, *CDKN1B* mRNA upregulation might be partly responsible for the observed suppression of LNCaP cell growth. CDKN-1B ablation/downregulation enhanced the aggressive prostate carcinoma phenotype, and in general, decreased CDKN-1B amount can be detected in various cancers with poor outcome (Roy *et al.* 2008; Borriello *et al.* 2011). As mentioned above, Barnard *et al.* (2007) could show that another regulator of cell cycle progression, CDKN-1A (p21), is also downregulated in prostate cancer cells by exercise serum from healthy subjects. It will be interesting to unravel the relationship between CDKN-1B and the CCNE-1–CDK-2 complex in cancer cells under the influence of exercise, since we could show that *CDKN1B* mRNA levels are up- and *CCNE1* levels are downregulated by an exercise stimulus.

Unfortunately, the exercise-mediated downregulation of *E2F1* (transcriptional activator) and DNA polymerase ε subunit 2 (*POLE2*) seen in the gene expression screen could not be confirmed (data not shown).

Data validation in the 72 h-response group (Fig. 5*C*) revealed that *WNT10A* mRNA is still decreased, by both post-WB-EMS patient serum ($P = 0.117$) and EPS-conditioned medium ($P = 0.033$), hinting at a more prominent role of Wnt-10A in the exercise-mediated control of human prostate cancer cell growth.

We also found that mRNA expression of dual-specificity phosphatase 8 (*DUSP8*), a tyrosine phosphatase targeting several mitogen-activated protein kinases (MAPKs), is enhanced (post-WB-EMS serum $P = 0.097$; post-intervention serum WB-EMS *vs.* control group $P = 0.012$; EPS $P = 0.0076$). Abnormalities in MAPK signalling have been implicated in multiple human malignancies. Little is known about the physiological function of DUSP-8, with contradictory function(s) in cancer cells (Nunes-Xavier *et al.* 2011). Further analysis should specify if *DUSP8* mRNA upregulation in 'exercise-exposed' LNCaP cells is involved in their proliferation control.

A, the scheme shows the result from a cancer-associated gene expression screen (730 genes; GEO database GSE135541) in 'exercise-exposed' LNCaP cells based on NanoString technology (treatment either with post- *vs*. pre-intervention serum pools from WB-EMS ($n = 8$) or control prostate cancer patients ($n = 10$), or with EPS-conditioned human myotube medium *vs*. unconditioned medium (medium pool from 3 independent EPS treatments)). Normalized data (WB-EMS group post-intervention/pre-intervention serum and control group post-intervention/pre-intervention serum; EPS-conditioned/unconditioned medium) were compared and potentially regulated genes were selected for further analysis (refer to Table 4). A difference in gene expression ratio of >20% between EPS medium-treated and -untreated LNCaP cells, and between post- and pre-WB-EMS serum-treated cancer cells was selected as measure for significance. Additionally, gene expression in LNCaP cells stimulated with a post-intervention serum pool from control prostate cancer patients had to be unaffected or inversely regulated compared to the WB-EMS ratio. After a 24 h treatment with EPS-conditioned medium from human myotubes, 68 genes were up- and 37 genes were downregulated. Within these groups of genes, the expression of 4 genes was also increased by post-WB-EMS patient serum and the expression of 7 genes was decreased by WB-EMS. The stimulation with EPS medium for 72 h yielded 61 upregulated and 83 downregulated genes. Among these, the expression of 6 genes was also higher and the expression of 9 genes was lower after a post-WB-EMS patient serum incubation run in parallel. Genes of these intersections (potentially) controlling cancer cell growth and apoptosis were chosen for further analysis. *B*, expression of genes involved in cancer cell proliferation and cell death was measured in serum-starved LNCaP cells after stimulation for 24 h at 37°C with either WB-EMS exercise or control serum from advanced prostate cancer patients, or EPS-conditioned medium pool from human myotubes. mRNA expression levels were detected using qRT-PCR and are depicted as fold change (relative to the expression in cells treated with post-intervention control serum pool). *CASP3* and *CASP7* are greyed out since they did not fit the initial selection criteria after the gene expression array, but significant changes in mRNA expression could be detected in the validation analysis using qRT-PCR. *C*, as described in *B*, LNCaP gene expression was quantified after 72 h treatment with post-WB-EMS serum or EPS-conditioned medium. Graphs depict the means (SD) of 5–6 independent experiments for patient serum incubation and 3 independent experiments for EPS medium treatment. *D*, immunoblot analysis of PARP cleavage after WB-EMS serum stimulation. LNCaP cells were serum-deprived for 18 h and treated with control and WB-EMS serum for 96 h. As a control for cell death-associated PARP cleavage, cells were treated with 10 μg ml^{-1} cycloheximide (CHX) for 24 h. Cell lysates were analysed using α-cleaved (cl.) PARP and PARP immunoblotting. Signal intensities from 4 independent experiments were quantified (including background and protein loading; mean (SD), graph). *E*, LNCaP cells were serum-starved, incubated with EPS-conditioned or unconditioned myotube medium for 96 h, and analysed as described in *D*. The graph depicts densitometry results from 4 independent experiments (mean (SD)). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$; paired (for comparison of the serum effect post- *vs*. pre-WB-EMS; *B*, *C* and *D*) and unpaired (for comparison of the post-intervention serum effect of WB-EMS *vs*. control group, as well as for the EPS-conditioned medium effect; *B*, *C* and *E*) two-tailed Student's *t* test.

Table 4. Potential exercise-sensitive genes regulating prostate cancer cell proliferation and apoptosis after multiplex analysis

Gene	WB-EMS serum, post/pre (fold change)	Control serum, post/pre (fold change)	Myotube medium, EPS-conditioned/ unconditioned (fold change)
24 h			
CCNE1	−1.28	1.10	−1.23
CDKN1B	1.49	−1.28	1.27
E2F1	−1.31	1.02	−1.33
GHR	−1.23	1.26	−1.20
POLE2	−1.31	1.11	−1.42
WNT10A	−1.26	1.00	−1.26
72 h			
BID	1.39	−1.14	1.27
*CASP3**	1.24	−1.01	1.10
CASP7	1.33	−1.24	1.50
DUSP8	1.43	1.02	1.87
WNT10A	−1.43	1.00	−1.22

Fold change in gene expression after incubation with pre- and post-intervention patient serum or EPS-conditioned medium for 24 h or 72 h. *CASP3 did not fit our initial selection criteria (i.e. the ratio of EPS *vs.* untreated >20%), but could potentially be important.

We also detected that the mRNA expression level of the BH3 interacting-domain death agonist *BID* is elevated by treatment with post-WB-EMS serum for 72 h, with a significant difference between *BID* mRNA levels upon stimulation with post-intervention serum from the WB-EMS or control group ($P = 0.039$). In EPS medium-stimulated cells we detected a trend towards an upregulation of *BID*. Among others, BID is essential for initiation of apoptosis signalling; it engages all Bcl-2 pro-survival proteins and is therefore a potent cell killer (Willis & Adams, 2005). However, the significance of BID expression in malignancies is unclear (Song *et al.* 2010). *BID* mRNA upregulation upon exposure to exercise might have pro-apoptotic effects specifically on LNCaP cells, but further experiments are needed for clarification.

Additionally, we identified that caspase 7 (*CASP7*) mRNA expression is significantly increased under the described conditions (post- *vs.* pre-WB-EMS serum $P = 0.033$, post-intervention serum WB-EMS *vs.* control group $P = 0.054$; EPS $P = 0.0164$). Caspases, sequentially activated, play pivotal roles in apoptotic events. Down-regulation of, for example, Casp-7 was described in colon cancer samples compared with normal mucosa, and resistance to apoptosis in renal cancer cells correlated with loss/downregulation of, for example, Casp-7 and -3 levels (Kolenko *et al.* 1999; Palmerini *et al.* 2001). In our gene expression screen, caspase 3 (*CASP3*) mRNA was borderline increased upon stimulation with both post-WB-EMS serum and EPS-conditioned medium for 72 h (Table 4). Validation experiments corroborated this result (post- *vs.* pre-WB-EMS serum $P = 0.173$, post-intervention serum WB-EMS *vs.* control group $P = 0.051$; EPS $P = 0.044$; Fig. 5*C*), suggesting that *CASP3* mRNA upregulation might also contribute to exercise-induced apoptosis in prostate cancer cells. According to the literature, prostatic as well as breast and ovarian cancer cells show significant loss of Casp-3 expression (Winter *et al.* 2001; Devarajan *et al.* 2002). Confirmatory gene expression experiments shown in Fig. 5*B* revealed that *CASP3* and *CASP7* mRNA are also upregulated after shorter stimulation (24 h) with post-WB-EMS serum or EPS-conditioned medium (*CASP3*: post- *vs.* pre-WB-EMS serum $P = 0.099$; EPS $P = 0.019$, and *CASP7*: post-intervention serum WB-EMS *vs.* control group $P = 0.016$; EPS $P = 0.0004$), pointing towards a more dominant function of Casp-3 and -7 in exercise-mediated apoptosis/death of prostate cancer cells.

Cleavage of poly(ADP ribose) polymerase 1 (PARP) is a hallmark of apoptosis. This cell death substrate has a canonical Casp-3/7 cleavage site (Oliver *et al.* 1998; Boucher *et al.* 2012). In order to corroborate our gene expression results regarding Casp-3/7, we performed cleaved PARP immunoblot analysis on LNCaP cell lysates after stimulation with control and WB-EMS prostate cancer serum as well as with medium from untreated and EPS-treated myotubes (96 h; Fig. 5*D* and *E*). After densitometry analysis, we found only a minor trend towards an increased PARP cleavage after incubation with post-WB-EMS serum (Fig. 5*D*). However, stimulation of LNCaP cells with EPS myotube medium led to a significant, almost twofold enhancement in PARP cleavage (Fig. 5*E*, densitometry graph, $P = 0.011$).

We could speculate that the EPS-conditioned medium can exert 'cleaner', more specific effects compared to the medium plus serum from the patients, which contains potentially interfering compounds and factors from the anti-cancer therapy and/or other medication. This discrepancy in the cell death analysis became already apparent in the DNA fragmentation experiments, where fragmentation was more pronounced upon EPS medium (Fig. 4*D*) than with medium plus post-WB-EMS serum (Fig. 2*F*). The WB-EMS/EPS-mediated upregulation of *CASP3* and *CASP7* gene expression seems to correlate with the EPS-induced PARP cleavage. We propose a mechanistic link between muscle stimulation and cancer cell death, initiated by an exercise-induced muscle-derived player that can activate the caspase pathway via the regulation of *CASP3 and CASP7* mRNA expression. This, in turn, leads to an increased cleavage of the Casp-3/7 substrate PARP and ultimately to enhanced DNA fragmentation and cell death.

Taken together, we could show that exercise regulates growth of human cancer cells, regardless of their hormone

dependency. For the first time, we demonstrated that *in vivo* exercise in the form of WB-EMS during advanced cancer disease can inhibit cancer cell proliferation and concomitantly activate apoptotic events *in vitro*, and provide evidence that genes associated with cancer cell growth and viability are regulated by exercise. Our comparative approach using both patient sera and supernatant from myotube cultures following exercise revealed similar effects on cancer cell proliferation and apoptosis. Therefore, we can target the origin of the observed anti-cancer effect directly to muscle cells, suggesting that one or several myokines might be released during exercise (refer to Table 3; Karstoft & Pedersen, 2016). Future studies should aim for identification of (potentially) involved myokines. We are convinced that our data further support the application of adjuvant exercise as part of multimodal therapeutic concepts for cancer patients, also for physically weakened patients. Regular exercise not only stabilizes the physical and mental status of cancer patients, but also seems to actively battle cancer development and progression.

References

Aoi W, Naito Y, Takagi T, Tanimura Y, Takanami Y, Kawai Y, Sakuma K, Hang LP, Mizushima K, Hirai Y, Koyama R, Wada S, Higashi A, Kokura S, Ichikawa H & Yoshikawa T (2013). A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. *Gut* **62**, 882–889.

Barnard RJ, Leung PS, Aronson WJ, Cohen P & Golding LA (2007). A mechanism to explain how regular exercise might reduce the risk for clinical prostate cancer. *Eur J Cancer Prev* **16**, 415–421.

Borriello A, Bencivenga D, Criscuolo M, Caldarelli I, Cucciolla V, Tramontano A, Borgia A, Spina A, Oliva A, Naviglio S & Della Ragione F (2011). Targeting p27Kip1 protein: its relevance in the therapy of human cancer. *Expert Opin Ther Targets* **15**, 677–693.

Boucher D, Blais V & Denault JB (2012). Caspase-7 uses an exosite to promote poly(ADP ribose) polymerase 1 proteolysis. *Proc Natl Acad Sci U S A* **109**, 5669–5674.

Brown JC & Gilmore LA (2020). Physical activity reduces the risk of recurrence and mortality in cancer patients. *Exerc Sport Sci Rev* **48**, 67–73.

Chu IM, Hengst L & Slingerland JM (2008). The Cdk inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. *Nat Rev Cancer* **8**, 253–267.

Dehkhoda F, Lee CMM, Medina J & Brooks AJ (2018). The growth hormone receptor: mechanism of receptor activation, cell signaling, and physiological aspects. *Front Endocrinol* **9**, 35.

Dethlefsen C, Hansen LS, Lillelund C, Andersen C, Gehl J, Christensen JF, Pedersen BK & Hojman P (2017). Exercise-induced catecholamines activate the hippo tumor suppressor pathway to reduce risks of breast cancer development. *Cancer Res* **77**, 4894–4904.

Devarajan E, Sahin AA, Chen JS, Krishnamurthy RR, Aggarwal N, Brun AM, Sapino A, Zhang F, Sharma D, Yang XH, Tora AD & Mehta K (2002). Down-regulation of caspase 3 in breast cancer: a possible mechanism for chemoresistance. *Oncogene* **21**, 8843–8851.

Devin JL, Hill MM, Mourtzakis M, Quadrilatero J, Jenkins DG & Skinner TL (2019). Acute high intensity interval exercise reduces colon cancer cell growth. *J Physiol* **597**, 2177–2184.

Dieli-Conwright CM, Parmentier JH, Sami N, Lee K, Spicer D, Mack WJ, Sattler F & Mittelman SD (2018). Adipose tissue inflammation in breast cancer survivors: effects of a 16-week combined aerobic and resistance exercise training intervention. *Breast Cancer Res Treat* **168**, 147–157.

Filipovic A, Kleinoder H, Dormann U & Mester J (2012). Electromyostimulation—a systematic review of the effects of different electromyostimulation methods on selected strength parameters in trained and elite athletes. *J Strength Cond Res* **26**, 2600–2614.

Gannon NP, Vaughan RA, Garcia-Smith R, Bisoffi M & Trujillo KA (2015). Effects of the exercise-inducible myokine irisin on malignant and non-malignant breast epithelial cell behavior in vitro. *Int J Cancer* **136**, E197–E202.

Goh J, Niksirat N & Campbell KL (2014). Exercise training and immune crosstalk in breast cancer microenvironment: exploring the paradigms of exercise-induced immune modulation and exercise-induced myokines. *Am J Transl Res* **6**, 422–438.

Hoffmann C & Weigert C (2017). Skeletal muscle as an endocrine organ: the role of myokines in exercise adaptations. *Cold Spring Harb Perspect Med* **7**, a029793.

Hojman P, Dethlefsen C, Brandt C, Hansen J, Pedersen L & Pedersen BK (2011). Exercise-induced muscle-derived cytokines inhibit mammary cancer cell growth. *Am J Physiol Endocrinol Metab* **301**, E504–E510.

Hsu RJ, Ho JY, Cha TL, Yu DS, Wu CL, Huang WP, Chu P, Chen YH, Chen JT & Yu CP (2012). WNT10A plays an oncogenic role in renal cell carcinoma by activating WNT/β-catenin pathway. *PLoS One* **7**, e47649.

Karstoft K & Pedersen BK (2016). Skeletal muscle as a gene regulatory endocrine organ. *Curr Opin Clin Nutr Metab Care*. **19**, 270–275.

Kemmler W, Engelke K & von Stengel S (2012). Effects of whole-body-electromyostimulation on sarcopenia in lean, elderly sedentary women. The TEST-III Study. *Deut Z Sportmed* **63**, 343–350.

Kemmler W, Schliffka R, Mayhew JL & von Stengel S (2010). Effects of whole-body electromyostimulation on resting metabolic rate, body composition, and maximum strength in postmenopausal women: the Training and ElectroStimulation Trial. *J Strength Cond Res* **24**, 1880–1887.

Kemmler W & von Stengel S (2013). Whole-body electromyostimulation as a means to impact muscle mass and abdominal body fat in lean, sedentary, older female adults: subanalysis of the TEST-III trial. *Clin Interv Aging* **8**, 1353–1364.

Kolenko V, Uzzo RG, Bukowski R, Bander NH, Novick AC, Hsi ED & Finke JH (1999). Dead or dying: necrosis versus apoptosis in caspase-deficient human renal cell carcinoma. *Cancer Res* **59**, 2838–2842.

Li P, Liu W, Xu Q & Wang C (2017). Clinical significance and biological role of Wnt10a in ovarian cancer. *Oncol Lett* **14**, 6611–6617.

Malumbres M & Barbacid M (2009). Cell cycle, CDKs and cancer: a changing paradigm. *Nat Rev Cancer* **9**, 153–166.

Nakamura Y, Nawata M & Wakitani S (2005). Expression profiles and functional analyses of Wnt-related genes in human joint disorders. *Am J Pathol* **167**, 97–105.

Nedachi T, Fujita H & Kanzaki M (2008). Contractile C2C12 myotube model for studying exercise-inducible responses in skeletal muscle. *Am J Physiol Endocrinol Metab* **295**, E1191–E1204.

Ngo TH, Barnard RJ, Tymchuk CN, Cohen P & Aronson WJ (2002). Effect of diet and exercise on serum insulin, IGF-I, and IGFBP-1 levels and growth of LNCaP cells *in vitro* (United States). *Cancer Causes Control* **13**, 929–935.

Nikolic N, Bakke SS, Kase ET, Rudberg I, Flo Halle I, Rustan AC, Thoresen GH & Aas V (2012). Electrical pulse stimulation of cultured human skeletal muscle cells as an in vitro model of exercise. *PLoS One* **7**, e33203.

Nikolic N, Gorgens SW, Thoresen GH, Aas V, Eckel J & Eckardt K (2017). Electrical pulse stimulation of cultured skeletal muscle cells as a model for in vitro exercise–possibilities and limitations. *Acta Physiol* **220**, 310–331.

Nunes-Xavier C, Roma-Mateo C, Rios P, Tarrega C, Cejudo-Marin R, Tabernero L & Pulido R (2011). Dual-specificity MAP kinase phosphatases as targets of cancer treatment. *Anticancer Agents Med Chem* **11**, 109–132.

Oliver FJ, de la Rubia G, Rolli V, Ruiz-Ruiz MC, de Murcia G & Murcia JM (1998). Importance of poly(ADP-ribose) polymerase and its cleavage in apoptosis. Lesson from an uncleavable mutant. *J Biol Chem* **273**, 33533–33539.

Ornish D, Weidner G, Fair WR, Marlin R, Pettengill EB, Raisin CJ, Dunn-Emke S, Crutchfield L, Jacobs FN, Barnard RJ, Aronson WJ, McCormac P, McKnight DJ, Fein JD, Dnistrian AM, Weinstein J, Ngo TH, Mendell NR & Carroll PR (2005). Intensive lifestyle changes may affect the progression of prostate cancer. *J Urol* **174**, 1065–1069; discussion 1069–1070.

Palmerini F, Devilard E, Jarry A, Birg F & Xerri L (2001). Caspase 7 downregulation as an immunohistochemical marker of colonic carcinoma. *Hum Pathol* **32**, 461–467.

Piccirillo R (2019). Exercise-induced myokines with therapeutic potential for muscle wasting. *Front Physiol* **10**, 287.

Raschke S, Eckardt K, Bjorklund Holven K, Jensen J & Eckel J (2013). Identification and validation of novel contraction-regulated myokines released from primary human skeletal muscle cells. *PLoS One* **8**, e62008.

Roy S, Singh RP, Agarwal C, Siriwardana S, Sclafani R & Agarwal R (2008). Downregulation of both p21/Cip1 and p27/Kip1 produces a more aggressive prostate cancer phenotype. *Cell Cycle* **7**, 1828–1835.

Rundqvist H, Augsten M, Stromberg A, Rullman E, Mijwel S, Kharaziha P, Panaretakis T, Gustafsson T & Ostman A (2013). Effect of acute exercise on prostate cancer cell growth. *PLoS One* **8**, e67579.

Scheler M, Irmler M, Lehr S, Hartwig S, Staiger H, Al-Hasani H, Beckers J, de Angelis MH, Haring HU & Weigert C (2013). Cytokine response of primary human myotubes in an in vitro exercise model. *Am J Physiol Cell Physiol* **305**, C877–C886.

Schink K, Herrmann HJ, Schwappacher R, Meyer J, Orlemann T, Waldmann E, Wullich B, Kahlmeyer A, Fietkau R, Lubgan D, Beckmann MW, Hack C, Kemmler W, Siebler J, Neurath MF & Zopf Y (2018*a*). Effects of whole-body electromyostimulation combined with individualized nutritional support on body composition in patients with advanced cancer: a controlled pilot trial. *BMC Cancer* **18**, 886.

Schink K, Reljic D, Herrmann HJ, Meyer J, Mackensen A, Neurath MF & Zopf Y (2018*b*). Whole-body electromyostimulation combined with individualized nutritional support improves body composition in patients with hematological malignancies – a pilot study. *Front Physiol* **9**, 1808.

Schnyder S & Handschin C (2015). Skeletal muscle as an endocrine organ: PGC-1α, myokines and exercise. *Bone* **80**, 115–125.

Schwartz AL, de Heer HD & Bea JW (2017). Initiating exercise interventions to promote wellness in cancer patients and survivors. *Oncology* **31**, 711–717.

Song G, Chen GG, Hu T & Lai PB (2010). Bid stands at the crossroad of stress-response pathways. *Curr Cancer Drug Targets* **10**, 584–592.

Tarum J, Folkesson M, Atherton PJ & Kadi F (2017). Electrical pulse stimulation: an in vitro exercise model for the induction of human skeletal muscle cell hypertrophy. A proof-of-concept study. *Exp Physiol* **102**, 1405–1413.

von Stengel S, Kemmler W, Engelke K & Kalender WA (2012). Effect of whole-body vibration on neuromuscular performance and body composition for females 65 years and older: a randomized-controlled trial. *Scand J Med Sci Sports* **22**, 119–127.

Weiss-Messer E, Merom O, Adi A, Karry R, Bidosee M, Ber R, Kaploun A, Stein A & Barkey RJ (2004). Growth hormone (GH) receptors in prostate cancer: gene expression in human tissues and cell lines and characterization, GH signaling and androgen receptor regulation in LNCaP cells. *Mol Cell Endocrinol* **220**, 109–123.

Whitham M & Febbraio MA (2016). The ever-expanding myokinome: discovery challenges and therapeutic implications. *Nat Rev Drug Discov* **15**, 719–729.

Willis SN & Adams JM (2005). Life in the balance: how BH3-only proteins induce apoptosis. *Curr Opin Cell Biol* **17**, 617–625.

Winter RN, Kramer A, Borkowski A & Kyprianou N (2001). Loss of caspase-1 and caspase-3 protein expression in human prostate cancer. *Cancer Res* **61**, 1227–1232.

Zhang C, Zhu Q, Gu J, Chen S, Li Q & Ying L (2019). Down-regulation of CCNE1 expression suppresses cell proliferation and sensitizes gastric carcinoma cells to Cisplatin. *Biosci Rep* **39**, BSR20190381.

Zhao H, Wang J, Zhang Y, Yuan M, Yang S, Li L & Yang H (2018). Prognostic values of CCNE1 amplification and overexpression in cancer patients: a systematic review and meta-analysis. *J Cancer* **9**, 2397–2407.

Additional information

Data availability statement

The raw data are available in the GEO database at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE135541, reference number GSE135541.

Competing interests

None.

Author contributions

R.S., K.S. and Y.Z. were involved in all aspects of the study: (1) conception or design of the work, (2) acquisition, analysis or interpretation of data for the work and (3) drafting the work or revising it critically for important intellectual content. S.S., W.D., D.R., O.F., H.J.H. and M.F.N. were involved in (2) and (3). All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This research work was supported by the H.W. & J. Hector Foundation, Weinheim, Germany.

Acknowledgements

Open access funding enabled and organized by Projekt DEAL.

Keywords

apoptosis, cancer cells, colorectal cancer, electric pulse stimulation, exercise, gene expression, physical activity, proliferation, prostate cancer, whole-body electromyostimulation

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Statistical Summary Document