ANTICANCER RESEARCH *39*: 4721-4728 (2019)
doi:10.21873/anticanres.13654

The Effects of Analgesics and Local Anesthetics on Gene Transcription Mediated by NFATc2 and Sp1 in Pancreatic Carcinoma

MANUELA MALSY, BERNHARD GRAF and ANIKA BUNDSCHERER

Department of Anesthesiology, University Medical Center Regensburg, Regensburg, Germany

Abstract. *Background/Aim: Recent research has identified the transcription factors NFATc2 and Sp1 as key regulators in the carcinogenesis of pancreatic carcinoma. This study aimed to examine the effect of clinically achievable dosages of analgesics including ketamine, s-ketamine, metamizole, and paracetamol as well as that of sufentanil, ropicavaine, and lidocaine on pancreatic carcinoma cells and the expression of NFATc2 and Sp1. Materials and Methods: The effects of analgesics on the expression of NFATc2 and Sp1 were investigated with immunoblotting. Cell proliferation was measured with the ELISA BrdU assay. Results: In PaTu8988t pancreatic carcinoma cells, 48 h stimulation with ketamine and s-ketamine significantly inhibited proliferation and decreased expression of NFATc2 in the nucleus. The addition of metamizole and lidocaine reduced proliferation of PaTu8988t cells after 48 h. Conclusion: New treatment concepts target specific signaling and transcription pathways. The extent to which drugs influence these mechanisms in pancreatic carcinoma cells needs to be investigated in future studies.*

Pancreatic adenocarcinoma is the fourth leading cause of cancer-related deaths worldwide (1). This type of cancer is known for its extremely poor long-term survival rates of 5 years which is only about 7% of all affected patients as well as for its almost identical incidence and mortality rates (2). The treatment of choice next to chemotherapy or radiation is surgical removal of the tumor (3). However, research carried out in the past few years has shown that the perioperative period is a particularly vulnerable phase in which tumor progression and metastasis are facilitated (4). Medication, surgery, and perioperative immunosuppression induce the constitutive activation of important signaling pathways and change the expression of transcription factors (5). Thus, it is hardly surprising that approximately 80% of affected patients show tumor recurrence even after surgery (2) and that the mean survival rate is reduced to approximately 9 months only (6).

Key elements in the carcinogenesis of pancreatic carcinoma are NFAT transcription factors (7). NFAT is the abbreviation of 'nuclear factor of activated T cells' that was first described by Shaw *et al.* in 1988 (8). NFATs control gene expression during activation and differentiation of T lymphocytes (9). In addition, these proteins are also expressed in a wide range of cells and tissue types and regulate genes involved in cell cycle, apoptosis, angiogenesis, and metastasis (10). As transcription factors, NFATs also control the expression of central genes involved in the control of growth and differentiation in pancreatic carcinoma (9). Furthermore, NFAT has been shown to interact with the transcription factor Sp1 (11).

It is still unclear to what extent the oncogenic transcription factors NFATc2 and Sp1 are influenced by agents given during the perioperative period for anesthesia or pain therapy.

The purpose of this study was to conduct an *in vitro* analysis of the impact of clinically achievable dosages of the analgesics ketamine, s-ketamine, metamizole, and paracetamol as well as of sufentanil, ropicavaine, and lidocaine on the pancreatic carcinoma cell lines PaTu8988t in dependency to NFATc2 and Sp1.

Materials and Methods

Cell lines. The human pancreatic cancer cell line PaTu8988t was obtained from Professor Ellenrieder (Philipps University of Marburg, Germany). PaTu8988t cells were maintained in Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Gallen, Switzerland) supplemented with 10% fetal calf serum (FCS) (Sigma-Aldrich) and 5% Myco Zap (Lonza Verviers SPRL, Verviers, Belgium). Cells were cultured in humidified CO_2 atmosphere (5%) at 37˚C and maintained in monolayer culture. Experiments were performed with cells at ~70-80% confluence.

Correspondence to: Manuela Malsy, MD, Department of Anesthesiology, University Medical Center Regensburg, Franz Josef Strauss Allee 11, 93053 Regensburg, Germany. Tel: +49 9419447801, Fax: +49 9419447802, e-mail: Manuela.Malsy@ukr.de

Key Words: Pancreatic carcinoma, cancer, analgesics, transcription factors, NFATc2, Sp1.

Reagents. Commercially available ropivacaine (Fagron, Barsbüttel, Germany), sufentanil (Sigma-Aldrich, St. Gallen, Switzerland), and lidocaine (Sigma-Aldrich) were used for this study. Ketamine and s-ketamine were purchased from Sigma-Aldrich (St. Gallen, Switzerland), metamizole from Fluka (München, Germany), and paracetamol from Merck (Darmstadt, Germany). Final concentrations were obtained by diluting drugs in standard growth media. All solutions were freshly prepared prior to use.

Subcellular fractionation and immunoblotting. For subcellular fractionation in nuclear and cytoplasmic cell fractions, cells were washed twice with cold DPBS, re-suspended in Puffer A (200 µl 1 M Hepes KOH, 100 µl 1 M KCl, 100 µl 1 mM EDTA, 10 µl 1 mM DTT, proteinase inhibitors, and 9.5 ml aqua dest) for 15 min and centrifuged at 3.600 rpm for 12 min. The supernatant (cytoplasmic part) was transferred into Eppendorf tubes, and the pellet (nuclear part) was dissolved in RIPAE buffer (5 ml Triton X100, 190 mg EDTA, 0.5 g SDS, 2.5 g Deoxycolid acid, 500 ml DPBS, proteinase inhibitors) for 15 min and centrifuged at 13,000 rpm for 30 min. Supernatants were transferred to new tubes and incubated on ice. For western blotting, 30 µg protein extracts were analyzed by SDS-PAGE and blotted onto nitrocellulose membrane. Membranes were washed in Tris-buffered saline (TBS) washing buffer (50 mM Tris-CI, 150 mM NaCl), incubated in blocking solution for 3 h and then incubated for 24 h with primary antibodies against NFATc2 (Cell Signaling, MA, USA), Sp1 (Cell Signaling), Lamin B (Santa Cruz, Dallas, TX, USA), and β-actin (Sigma-Aldrich). Following four washes with TBS, the membranes were incubated with peroxidase-conjugated secondary antibodies (Cell Signaling). Immunoreactive proteins were visualized by means of an enhanced chemiluminescence detection system (Western Blotting Detection Reagent, GE Healthcare, Chicago, IL, USA).

Cell proliferation. Quantification of cell proliferation was based on the measurement of BrdU incorporation during DNA synthesis. The test was performed according to the manufacturer's protocol (Cell proliferation ELISA BrdU, Roche, Basel, Switzerland). In brief, cells were incubated with 100 µl of the test compounds for 0, 24, 48 and 72 h. After 8, 32, or 56 h of incubation, cells were additionally treated with BrdU labeling solution for the last 16 h. The culture medium was removed, cells were fixed, and DNA was denatured. Afterwards, cells were incubated with Anti-BrdU-POD solution for 90 min, antibody conjugate was removed by flicking off and wells were rinsed with three washing cycles (PBS). Immune complexes were detected by means of TMB substrate (3,3,5,5-Tetramethylbenzidine Liquid Substarte) for 15 min and quantified by measuring the absorbance at 405 nm and 490 nm. All tests were performed in duplicates with eight wells per treatment group and repeated three times.

Statistical analysis. Data are presented as mean±SD. The non-parametric Mann-Whitney *U*-test was used for the statistical evaluation of the data. *p*-Values of <0.05 were considered significant. IBM SPSS Statistics (Vs. 22; IBM, NY, USA) and Excel Vs. 2013 (Microsoft, Redmond, WA, USA) packages were employed for statistical analysis.

Results

Effects of ketamine and s-ketamine. PaTu8988t pancreatic cancer cells were stimulated with 5 µM ketamine or 5 µM s-ketamine for 0, 24, 48 and 72 h. Cell proliferation was significantly decreased after 48 h stimulation with ketamine and s-ketamine (Figure 1a).

The protein expression of NFATc2 and Sp1 in PaTu8988t pancreatic carcinoma cells was assayed by western blotting. Cells treated with FCS showed presence of NFATc2 in the nucleus. Twenty-four and 48 h stimulation with 5µM ketamine or 5 µM s-ketamine reduced the expression levels of NFATc2 in the nucleus of pancreatic carcinoma cells. Concurrently, stimulation with ketamine increased the expression of NFATc2 in the cytoplasm. After 72 h stimulation, expression of NFATc2 in the cell nucleus was significantly increased again. The expression levels of Sp1 remained unchanged, and Lamin B and β-actin served as a loading control (Figure 1b).

Effect of metamizole and paracetamol. PaTu8988t pancreatic cancer cells were stimulated with 75 µM metamizole, 100 µM paracetamol, or a combination of 75 µM metamizole and 100 µM paracetamol for 0, 24, 48 and 72 h each (Figure 2a, b and c). Proliferation was significantly inhibited after stimulation with metamizole for 48 h. Paracetamol and the combination of 75 µM metamizole and 100 µM paracetamol did not significantly affect cell growth (Figure 2a).

Western blot analysis of samples incubated in metamizole, paracetamol, or the combination of metamizole and paracetamol showed no change in the expression of NFATc2 or Sp1. Column 3 depicts the endogenous expression of Lamin B and column 4 the expression of β-actin that served as a loading control (Figure 2b and c).

Effect of ropivacaine, lidocaine and sufentanil. PaTu8988t pancreatic cancer cells were stimulated with 5 µM ropivacaine, 1.5 nM sufentanil, 5 µM ropivacaine, 1.5 nM sufentanil or 10 µM lidocaine for 0, 24, 48 and 72 h each (Figure 3a, 3b, 3c). Stimulation with 10 µM lidocaine for 48 h resulted in a slight but statistically significant decrease in cell proliferation of PaTu8988t cell (Figure 3a). Stimulation for 0, 24 or 72 h or use of the other test substances did not change the proliferation rate in comparison to untreated control (Figure 3a).

Western blot analysis did not yield any effects at the protein levels of NFATc2 and Sp1 after stimulation for 0, 24, 48 or 72 h. Furthermore, subcellular localization of NFATc2 remained unchanged. Columns 3 and 4 depict the loading control (Figure 3b and c).

Discussion

Medications given in the context of anesthesia and postoperative pain therapy of pancreatic carcinoma, such as ketamine, s-ketamine, metamizole, and paracetamol but also sufentanil, ropicavaine, and lidocaine, exert different effects and their administration requires careful consideration. The purpose of this study was to examine the effect of clinically

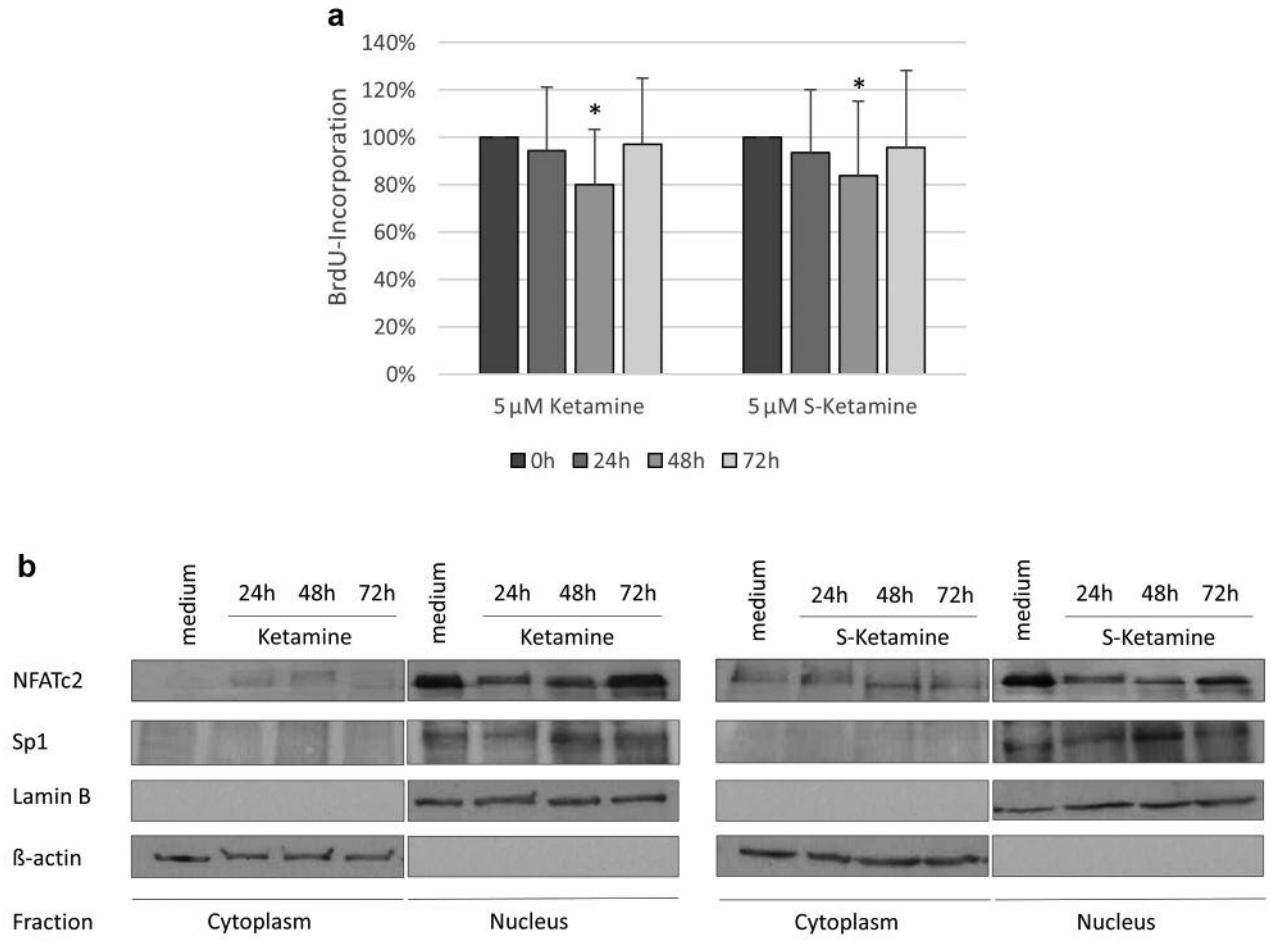

Figure 1. *The effect of ketamine and s-ketamine on the proliferation (a) and endogenous expression of NFATc2, Sp1, Lamin B, and β-actin (b) in PaTu8988t pancreatic cancer cell lines after incubation for 0, 24, 48 and 72 h. The proliferation rate was determined by means of proliferation BrdU assays. 100% corresponds to untreated control. *p<0.05 in comparison to untreated control.*

relevant drug concentrations to facilitate the transfer of experimental data to clinical practice.

As an NMDA receptor antagonist, ketamine in narcotic dosages causes profound analgesia; thus, this drug is not only successfully used for the management of cancer pain and opioid-refractory pain but also in preventive pain therapy (12, 13). Plasma concentrations achievable in clinical settings range between 0.1 and 0.42 μg/ml (≈2.3-9.5 μM) (14). The main impact of ketamine and s-ketamine is based on the non-competitive blockade of the NMDA receptor complex. In the process, ketamine as well as its racemate s-ketamine bind to the binding site of phencyclidine (PCP) inside the NMDA channel, thus inhibiting the effect of NMDA antagonists (15). This process results in the reduction of the intracellular calcium concentration and in the inactivation of Ca2+-dependent cytosolic guanylate cyclase (16). Several studies have described the influx of calcium into the cell as a key trigger or regulator of cellular processes relevant to tumor progression including proliferation and apoptosis (17). Interestingly, 24h and 48h stimulation with ketamine and s-ketamine initially reduced the levels of NFAT transcription factors in the nucleus of pancreatic carcinoma cells PaTu8988t; at the same time, stimulation with ketamine increased the levels of NFATc2 in the cytoplasm.

In dormant inactive cells, NFATc2 proteins are present in the cytoplasm in a phosphorylated form. The proteins have only a low affinity for DNA (18) and are activated by stimulation with FCS. The resulting intracellular increase in calcium activates the protein serine/threonine phosphatase calcineurin, which can thus bind to the PxIxIT motifs located at the N-terminal of NFAT proteins. As a result, NFAT proteins are dephosphorylated on 13 serine residues (19). Due to the resulting conformational change, NFAT proteins are translocated into the nucleus and bind to specific DNA-

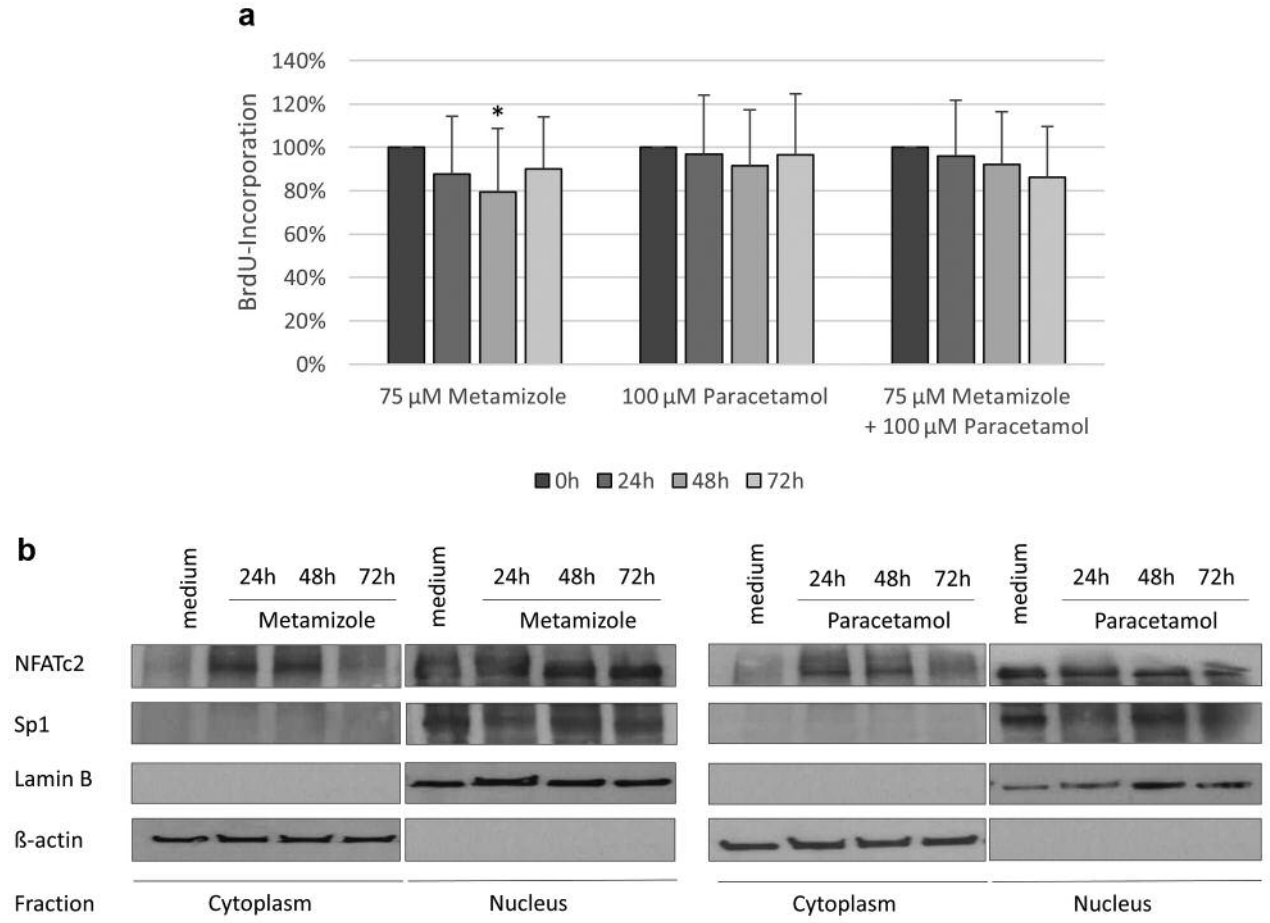

Figure 2. *The effect of metamizole, paracetamol, and the combination of metamizole and paracetamol on PaTu8988t pancreatic carcinoma cell proliferation after stimulation for 0, 24, 48, and 72 h. a: The proliferation rate was detected by means of BrdU uptake. (*) indicates statistical significance at p<0.05 compared to untreated control. b, c: Immunoblot analysis of the endogenous expression of NFATc2, Sp1, Lamin B, and β-actin after stimulation PaTu8988t pancreatic cancer cells for 0, 24, 48 and 72 h with metamizole, paracetamol, (b) and the combination of metamizole and paracetamol (c).*

binding sequences (GGAAA) (20). NFAT proteins eventually interact with other transcription factors and exert their carcinogenic effect (21). Both 24 and 48 h stimulation with ketamine or s-ketamine seem to be able to inhibit this protein activation cascade. NFAT localizes into the cytoplasm in a dormant stage, and cell proliferation decreases after stimulation for 48 h.

The pyrazolone derivative metamizole (dipyrone) and the aminophenol derivative paracetamol (acetaminophen) are non-acidic, non-opioid analgesics (22, 23). The administration of these drugs is a key element of the WHO's cancer pain ladder (24) and an important part of postoperative analgesia (25). Oral administration of 1g metamizole results in a maximum plasma concentration of 17.3±7.5 mg/l (≈50-75 µM metamizole) and the intravenous injection of 1g paracetamol results in a plasma concentration of 95±36 µM (26, 27). According to the literature, metamizole and paracetamol primarily inhibit cyclooxygenase activity (COX), thus influencing prostaglandin synthesis as the central regulator of inflammation and inhibiting the transformation of arachidonic acid into endoperoxide, the precursor of prostaglandin, thromboxane A2, and prostacyclin (28, 29).

In this study, proliferation of PaTu8988t pancreatic carcinoma cells was inhibited by the administration of metamizole. Paracetamol has been shown to have a slight but significant anti-proliferative effect in PaTu8988t and Panc-1 pancreatic carcinoma cells in an earlier study (30). Stimulation with metamizole and paracetamol did not change the levels of expression of NFATc2 or Sp1.

It has recently been shown that COX-2 inhibitors increase Sp1 protein degradation (31). Tolfenaminic acid is a non-steroidal anti-inflammatory drug (NSAID) that additionally

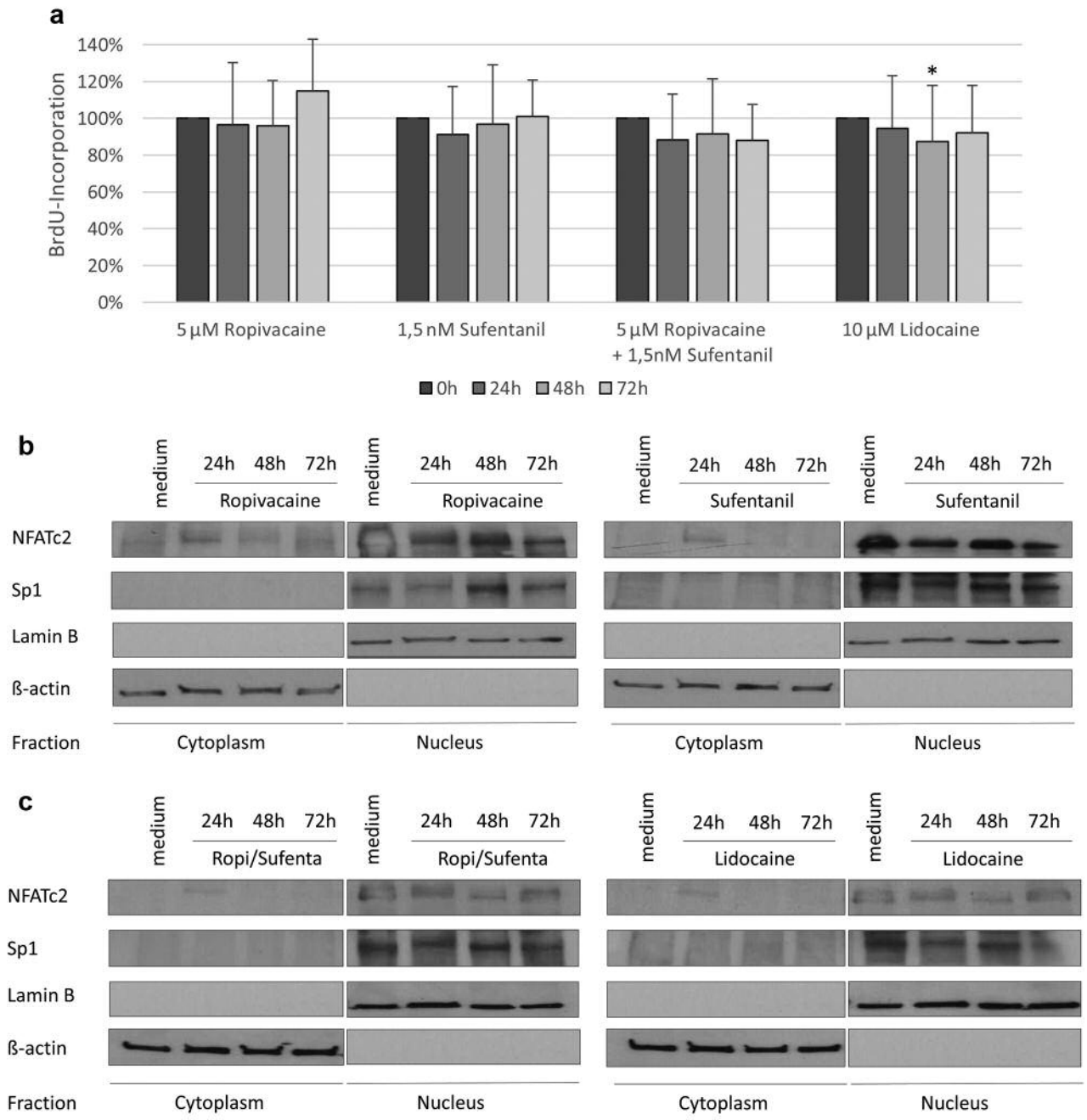

Figure 3. *The effects of ropivacaine, sufentanil, the combination of ropivacaine and sufentanil, and lidocaine on proliferation of PaTu8988t pancreatic carcinoma cells in vitro. a: Cell proliferation was quantified by measuring BrdU incorporation. (*) indicates statistical significance at $p<0.05$ compared to untreated control. b, c: Western blot analysis of the endogenous expression of NFATc2, Sp1, Lamin B, and β-actin after stimulation of PaTu8988t pancreatic cancer cells for 0, 24, 48 and 72 h with ropivacaine, sufentanil (b), the combination of ropivacaine, sufentanil, and lidocaine (c).*

activates the degradation of Sp1, Sp3, and Sp4 and decreases the expression of several Sp regulated growth-promoting, angiogenic, survival, and inflammatory gene products (32). These characteristics are of particular significance given the long existing assumption that Sp1 expression is a key factor in tumor development, growth, and metastasis. In some types of cancers, Sp1 overexpression is associated with poor survival (33).

Ropivacaine and lidocaine are amide local anesthetics (34) that block voltage-gated sodium channels of neuronal axons. The local anesthetics bind inside of the inactivated sodium channel, thus impeding the fast sodium influx into the cell that is important for depolarization (35). The conduction of stimuli in the nerve is inhibited, thus stopping the transmission of pain (36). Several studies have shown that plasma concentrations of 0.61-4 μg/ml (≈1.6-10.9 μM) are achievable with ropivacaine and 1-5 μg/ml (≈2.3-11.5 μM) with lidocaine (37, 38). In peridural anesthesia, ropivacaine is often combined with the opioid sufentanil (39). As a pure agonist, sufentanil binds to the opioid receptors of the nervous system (40) and has been proven to improve the quality of analgesia. In peridural anesthesia, the addition of opioids to local anesthetics results in a faster onset of effects and reduces the dosage of the individual drugs (41). Plasma concentrations achievable with sufentanil are 0.40±0.14 ng/ml (≈1.5 nM) (42). Similar effects can also be observed following intravenous injections of lidocaine in large abdominal surgical interventions in contrast to singular general anesthesia. The reduction in peri- and postoperative pain significantly decreases the requirement of anesthetics and opioid analgesics (43). In our study, the administration of lidocaine decreased proliferation after 48 h, but ropivacaine, sufentanil, and lidocaine had no effect on the expression of the transcription factors NFATc2 and Sp1 in pancreatic carcinoma cells.

Conclusion

Pancreatic adenocarcinoma is one of the most aggressive cancers. Its oncogenic potential is mainly marked by extremely fast growth trigged by the activation of important signaling cascades during vulnerable phases. Thus, new therapeutic concepts also target the efficient modulation of specific signaling and transcription pathways. A wide variety of inhibitors is being investigated in the context of preclinical studies or is currently being established in clinical practice (34, 35). One possible novel therapeutic concept for pancreatic carcinoma cells is the inhibition of the interaction between NFATc2 and Sp1. The extent to which medication influences mechanisms in vulnerable phases of pancreatic carcinoma needs to be investigated in future studies. The basis of novel therapeutic approaches to any disease is detailed knowledge of the carcinogenesis and profound molecular and biological understanding of the mechanisms.

Conflicts of Interest

The Authors declare that they have no competing interests regarding this study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

All Authors have made substantial contributions to the conception, design, analysis, and the interpretation of this research article. They have been involved in the critical revision of the manuscript with regard to important intellectual content. All Authors have given their final approval for the version to be published 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.

Acknowledgements

The Authors thank Sigrid Bamberger, Regina Lindner, Gabriele Bollwein, Marion Schindler, Ruth Spaeth and Michael Gruber for technical assistance. The Authors would also like to thank Monika Schoell for linguistic support.

References

1 Vincent A, Herman J, Schulick R, Hruban RH and Goggins M: Pancreatic cancer. Lancet *378*: 607-620, 2011. PMID: 21620466. DOI: 10.1016/S0140-6736(10)62307-0
2 Pancreatic cancer facts 2016. Pancreatic cancer action network. Business Search, California Secretary of State.
3 Schneider G, Siveke JT, Eckel F and Schmid RM: Pancreatic cancer: basic and clinical aspects. Gastroenterology *128*: 1606-1625, 2005. PMID: 15887154. DOI: 10.1053/j.gastro.2005.04.001
4 Gottschalk A, Sharma S and Ford J: Review article: the role of the perioperative period in recurrence after cancer surgery. Anesth Analg *110*: 1636-1643, 2010. PMID:
5 20435944. DOI: 10.1213/ANE.0b013e3181de0ab6
6 Snyder GL and Greenberg S: Effect of anaesthetique and other perioperative factors on cancer recrurrence. Br J Anaesth *105*: 106-115, 2010. PMID: 20627881. DOI: 10.1093/bja/aeq164
7 Hidalgo M: Pancreatic cancer. N Engl J Med *362*: 1605-1617, 2010. PMID: 20427809. DOI: 10.1056/NEJMra0901557
8 König A, Fernandez-Zapico ME and Ellenrieder V: Primers on molecular pathways--the NFAT transcription pathway in pancreatic cancer. Pancreatology *10*: 416-422, 2010. PMID: 20720442. DOI: 10.1159/000315035
9 Shaw JP, Utz PJ, Durand DB, Toole JJ, Emmel EA and Crabtree GR: Identification of a putative regulator of early T cell activation genes. Science *241*: 202-205, 1988. PMID: 20962265.
10 Viola JP, Carvalho LD, Fonseca BP and Teixeira LK: NFAT transcription factors: from cell cycle to tumor development. Braz J Med Biol Res *38*: 335-344, 2005. PMID: 15761612. DOI: 10.1590/s0100-879x2005000300003
11 Mognol GP, Carneiro FR, Robbs BK, Faget DV and Viola JP: Cell cycle and apoptosis regulation by NFAT transcription factors: new roles for an old player. Cell Death Dis *7*: 1-13, 2016. PMID: 27100893. DOI: 10.1038/cddis.2016.97

12 Malsy M, Graf B and Almstedt K: Interaction between NFATc2 and the transcription factor Sp1 in pancreatic carcinoma cells PaTu 8988t. BMC Mol Biol *18*: 20, 2017. PMID: 28774282. DOI: 10.1186/s12867-017-0097-9
13 Bredlau AL, Thakur R, Korones DN and Dworkin RH: Ketamine for Pain in Adults and Children with cancer: a systematic Review and Synthesis of the Literature. Pain med *14*: 1505-1507, 2013. PMID: 23915253. DOI: 10.1111/pme.12182
14 Tawfic QA: A review of the use of ketamine in pain management. J Opioid Manag *9*: 379-388, 2013. PMID: 24353050. DOI: 10.5055/jom.2013.0180
15 Hameln. Fachinformation Ketamin. 009930-24181: 1-4, 2015.
16 Zgaia AO, Irimie A, Sandesc D, Vlad C, Lisencu C, Rogobete A and Achimas-Cadariu P: The role of ketamine in the treatment of chronic cancer pain. Clujul Med *88*: 457-461, 2015. PMID: 26733743. DOI: 10.15386/cjmed-500
17 Kress HG: Mechanisms of action of ketamine. Anaesthesist *46*: 8-19, 1997. PMID: 9163283
18 Monteith G, Davis F and Roberts-Thomson S: Calcium channels and pumps in cancer: changes and consequences. J Biol Chem *287*: 31666-31673, 2012. PMID: 22822055. DOI: 10.1074/jbc.R112.343061
19 Viola JPB, Carvalho LDS, Fonseca BPF and Teixeira LK: NFAT transriptions factors: from cell cycle to tumor development. Braz J Med Biol Res *38*: 335-344, 2014. PMID: 15761612. DOI: 10.1590/s0100-879x2005000300003
20 Im S and Rao A: Activation and Deactivation of gene expression by Ca/ Calcineurin-NFAT-mediates Signaling. Mol Cells *18*: 1-9, 2004. PMID: 15359117.
21 Hogan PG, Chen L, Nardone J and Rao A: Transcriptional regulation by calcium, calcineurin and NFAT. Genes & Development *17*: 2205-2232, 2003. PMID: 12975316. DOI: 10.1101/gad.1102703
22 Malsy M, Almstedt K and Graf B: The active role of the transcription factor Sp1 in NFATc2-mediated gene regulation in pancreatic cancer. BMC Biochemistry *20*: 2, 2019. PMID: 30696421. DOI: 10.1186/s12858-019-0105-4
23 Jasiecka A, Maślanka T and Jaroszewski JJ: Pharmacological characteristics of metamizole. Pol J Vet Sci *17*: 207-214, 2014. PMID: 24724493
24 Graham GG and Scott KF: Mechanism of action of paracetamol. Am J Ther 12: 46-55, 2005. PMID: 15662292.
25 Krome S: Cancer pain management: the WHO's analgesic ladder as guideline. Dtsch Med Wochenschr *136*: 94-96, 2011. PMID: 21960376. DOI: 10.1055/s-0031-1292071
26 S3-Leitlinie zum exokrinen Pankreaskarzinom, Kurzversion 1.0 – Oktober 2013, AWMF-Registernummer: 032/010OL, 2013.
27 Sanofi aventis. Fachinformation Novalgin 001511-D725: 1-7, 2011.
28 Holmer Pettersson P, Jakobsson J and Owall A: Plasma concentration following repeated rectal or intravenous administration of paracetamol after heart surgery. Acta Anaesthesiol Scand *60*: 673-677, 2006. PMID: 16987360. DOI: 10.1111/j.1399-6576.2006.01043.x
29 Pierre SC, Schmidt R, Brenneis C, Michaelis M, Geisslinger G and Scholich K: Inhibition of cyclooxygenases by dipyrone. Br J Pharmacol *151*: 494-503, 2007. PMID: 17435797. DOI: 10.1038/sj.bjp.0707239
30 Graham GG, Davies MJ, Day RO, Mohamudally A and Scott KF: The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. Inflammopharmacology *21*: 201-232, 2013. PMID: 23719833. DOI: 10.1007/s10787-013-0172-x
31 Malsy M, Graf B and Bundscherer A: Effects of metamizole, MAA, and paracetamol on proliferation, apoptosis, and necrosis in the pancreatic cancer cell lines PaTu 8988t and Panc-1. BMC Pharacol Toxicol *18*: 77, 2017. PMID: 29208039. DOI: 10.1186/s40360-017-0185-y
32 Abdelrahim M and Safe S: Cyclooxygenase-2 inhibitors decrease vascular endothelial growth factor expression in colon cancer cells by enhanced degradation of Sp1 und Sp4 proteins. Mol Pharmacol *68*: 317-329, 2005. PMID: 15883203. DOI: 10.1124/mol.105.011825
33 Pathi S, Li X and Safe S: Tolfenamic acid inhibits colon cancer cell and tumor growth and induces degradation of specificity protein (Sp) transcription factors. Mol Carcinog *1*: 53-61, 2014. PMID: 23670891. DOI: 10.1002/mc.22010
34 Abdelrahim M, Baker CH, Abbruzzese JL and Safe S: Tolfenamic acid and pancreatic cancer growth, angiogenesis, and Sp protein degradation. J Natl Cancer Inst *98*: 855-868, 2006. PMID: 16788159. DOI: 10.1093/jnci/djj232
35 Moore PA and Hersh EV: Local anesthetics: pharmacology and toxicity. Dent Clin North Am *54*: 587-599, 2010. PMID: 20831923. DOI: 10.1016/j.cden.2010.06.015
36 Biscoping J and Bachmann-Mennenga MB: Local anesthetics from ester to isomer. Anasthesiol Intensivmed Notfallmed Schmerzther *35*: 285-292, 2000. PMID: 10858837. DOI: 10.1055/s-2000-324
37 Curatolo M: Regional anesthesia in pain management. Curr Opin Anaesthesiol *29*: 614-619, 2016. PMID: 27137511. DOI: 10.1097/ACO.0000000000000353
38 Cusato M, Allegri M, Niebel T, Ingelmo P, Broglia M, Braschi A and Regazzi M: Flip-flop kinetics of ropivacain during continuous epidural infusion influences its accumulation rate. Eur J Clin Pharmacol *67*: 399-406, 2011. PMID: 21079936. DOI: 10.1007/s00228-010-0927-x
39 Kahokehr A, Sammour T, Vather R, Taylor M, Stapelberg F and Hill AG: Systemic levels of local anaesthetics after intraperitoneal application – a systemic review. Anaesthesia and intensive care *38*: 613-638, 2010. PMID: 20715724. DOI: 10.1177/0310057X1003800404
40 Bachmann-Mennenga B, Veit G, Steinicke B, Biscoping J and Heesen M: Efficacy of sufentanil addition to ropivacaine epidural anaesthesia for caesarean section. Acta Anaesthesiol Scand *49*: 532-537, 2005. PMID: 15777302. DOI: 10.1111/j.1399-6576.2005.00657.x
41 Bujedo BM, Santos SG and Azpiazu AU: A review of epidural and intrathecal opioids used in the management of postoperative pain. J Opioid Manag *8*: 177-192, 2012. PMID: 22798178. DOI: 10.5055/jom.2012.0114
42 Gomar C and Fernandez C: Epidural analgesia-anaesthesia in obstetrics. Eur. J. Anaesthesiol *17*: 542-558, 2000. PMID: 11029122.
43 Hansdottir V, Woestenborghs R and Nordberg G: The cerebrospinal fluid and plasma pharmacokinetics of sufentanil after thoracic or lumbar epidural administration. Anesth. Analg *80*: 724-729, 1995. PMID: 7893025. DOI: 10.1097/00000539-199504000-00013
44 McCarthy GC, Megalla SA and Habib AS: Impact of intravenous lidocaine infusion on postoperative analgesia and

recovery from surgery: a systematic review of randomized controlled trials. Drugs *70*: 1149-1163, 2010. PMID: 20518581. DOI: 10.2165/10898560-000000000-00000

45 Novak K: Conference report--protein kinase inhibitors in cancer treatment: mixing and matching? Highlights of the keystone symposium on protein kinases and cancer; February 24-29, 2004; Lake Tahoe, California, USA. MedGenMed *6*: 25, 2004.

46 Huang ZQ and Buchsbaum DJ: Monoclonal antibodies in the treatment of pancreatic cancer. Immunotherapy *1*: 223-229, 2009. PMID: 20046965. DOI: 10.2217/1750743X.1.2.223

Received August 7, 2019
Revised August 15, 2019
Accepted August 18, 2019