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N-Nitrosomorpholine-induced oncocytic transformation in rat endocrine organs

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Abstract

Background N-Nitrosomorpholine (NMO) is one of the most common N-nitroso compounds. An oncocytic transformation has been demonstrated in renal tubules of NMO-treated rats. In our study, we aimed to investigate the potential transformation of oncocytic cells in 6 endocrine organs, i.e., thyroid, adrenal and pituitary glands, pancreas, testis, and bone, of NMO-exposed rats.

Methods Thirty male rats were born and raised. Fifteen of them were given a single dose of 320 mg NMO per kg body weight, dissolved in drinking water, by a gavage tube. At the end of 52 weeks, the animals in both series were killed. Right after the killing, 6 different endocrine organs (hypophysis, thyroid, pancreas, adrenal gland, bone [femur], and testicles) of each animal were excised.

Results There was no evidence of oncocytic cell development in the control group. In contrast, oncocytes were observed in 8 out of 13 NMO-treated rats: 2 in the adrenal sections, 1 in the thyroid sections, 3 in the pituitary sections, and 2 in the pancreas sections. The testicle and bone sections were completely normal.

Conclusions We showed that NMO induced an oncocytic change in pancreas, thyroid, pituitary, and adrenal glands. To date, no identified specific environmental risk factors that lead to an oncocytic transformation in endocrine glands have been reported previously. Given the increasing prevalence of endocrine-disrupting chemicals in the environment, personal care products, manufactured goods, and food sources, there is a need to advance our understanding of the pathological mechanisms underlying oncocytosis in endocrine organs.

Keywords Nitroso compounds, Oncocyte, Endocrine disrupting chemical, Endocrine disruptors, Endocrine gland neoplasms

Background

N-Nitroso compounds have become a potentially large source of exposure for human beings to chemical carcinogens in the recent years. N-Nitrosomorpholine (NMO) is one of the most common N-nitroso compounds, which has been frequently detected in the discharged water from sewage treatment plants, some cosmetics and toiletries, fine-cut chewing tobacco used for snuff-dipping and as an air pollutant in regions where tire-manufacturing and rubber factories are located [1–4]. This tumorigenic compound has been shown previously to be mainly hepatotoxic, inducing hepatocellular carcinoma

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[5, 6]. Moreover, NMO also triggered the development of neoplasms in the respiratory and upper digestive tracts, thyroid gland, brain, and spleen in various animal experiments [7–10].

An oncoocyte is an enlarged cell with abundant eosinophilic, granular cytoplasm, in which a large hyperchromatic nucleus exists [11]. This oncocyctic change is caused by proliferation and accumulation of mitochondria within the cell, and it has been mainly attributed to the alterations related to the mitochondrial DNA [12]. These mitochondrial anomalies are triggered by chronic inflammation secondary to aging or various chemical carcinogens such as NMO [13–15]. Various studies have demonstrated an oncocyctic transformation in renal tubules of NMO-treated rats [15–20]. In addition to that, germline mutations of the *Gene associated with Retinoid-Interferon-induced Mortality (GRIM)-19* have also been indicated as a possible etiological factor in the development of an oncocyctic tumor [21]. This metaplastic differentiation has been observed in various organs, such as thyroid, parathyroid, pituitary and adrenal glands, pancreas, kidney and paraganglia [13]. In our study, we aimed to investigate the potential transformation of oncocyctic cells in six endocrine organs, i.e., thyroid, adrenal and pituitary glands, pancreas, testis, and bone, of NMO-exposed rats.

Materials and methods

Characterization of N-nitrosomorpholine (NMO)

NMO was provided from MedChemExpress, New Jersey, USA; via a distributor located in our region, named Suarge Biotechnology Co., Ltd., Istanbul, Turkey. The purity and solubility of NMO were reported as 98.22% and ≥ 100 mg/mL at 19 °C, respectively. The appearance was solid < 29 °C and liquid ≥ 29 °C. The chemical could be stored at 4 °C for 2 years. It was also reported that NMO was light-sensitive and could be stable at room temperature for more than 14 days in neutral form in dark.

Animal experiments

Thirty male Sprague–Dawley rats (about 8-week old) weighing about 200 g were born and raised in the Experimental Medicine Research and Application Unit of our institution. Fifteen rats were given a single dose of 320 mg NMO per kg body weight, dissolved in drinking water, by a gavage tube. The amount of this chemical agent was determined according to a previously reported rat study, in which a single dose of 320 mg/kg NMO was administered to the animals [20]. The animals were placed in different cages in groups of 5 and maintained under constant conditions (20 ± 2 °C and 12 h light/dark cycle). The rest 15 rats, used as controls,

were also administered placebo by gavage in the same day and housed in groups of 5 in the same room with the others. Afterward, the rats were kept on a standard laboratory diet and tap water for 52 weeks (*ad-libitum*).

The maintenance period from the start of the experiment to the killing was based on various rat studies which revealed data on the time of appearance and incidence of oncocyctic lesions [10, 14, 17, 18]. Laboratory animals were cared for and fed in Eurostandard type IV cages (1354G EUROSTANDARD TYPE IV 598 × 380 × 200 mm, floor area: 1820 cm²) defined under the condition of “management and implementation instructions for the breeding and protection of animals used for experimental and other scientific parts”. Only health monitoring protocol was to make a daily inspection of rats’ behavior and record in case of any abnormality. No abnormal behavior was observed, even in the rats which were deceased during the experiment. In addition to that, observation of daily food and water consumption of the rats was made and no differentiation of any rat’s consumption from the others was observed.

At the end of 52 weeks, the animals in both series were killed using xylazine and ketamine intraperitoneal injections. All rats were killed between 9 a.m. and 1 p.m. in 2 consecutive days; NMO-treated group in the first day and controls in the second day. The main reason to kill 2 groups in 2 consecutive days within the similar daytime period was to control for the circadian rhythm differences between the groups. Right after the killing, six different endocrine organs (hypophysis, thyroid, pancreas, adrenal gland, bone [femur], and testicles) of each animal were excised. The only excluding criterion in this study was the development of mortality during the experiment.

Histopathological examination

Each organ was stored rapidly in a plastic sterile container full of 10% formaldehyde solution. Thereafter, tissue samples taken from the removed organs were fixed and embedded in paraffin for light microscopic examinations. Each of the organs was examined by serial sectioning. Section thicknesses were 3 μ m. Nine separate sections were made for each organ. The histological sections were stained with hematoxylin and eosin (H&E). The presence of oncocyctic cells in each tissue section was investigated under the microscope by two pathologists. The 1st, 5th, and 9th sections were examined. Presence of any oncocyctic cell was expressed as percentage. The average of the oncocyctic cell percentages in these three sections for each organ was

recorded as the oncocytic cell percentage specific to that organ.

Results

Mortality data

A total of three rats, two from the carcinogen-treated group and one from controls, were found deceased within this period, by the weeks 44, 50, and 48, respectively, after the beginning of the experiment, respectively. Diagnostic necropsy examinations of the deceased rats revealed normal postmortem changes; thus, no additional examination was performed.

Histopathological outcome

Six endocrine organs (hypophysis, thyroid, pancreas, adrenal gland, bone, and testicles) of 27 rats (13

NMO-treated and 14 controls) were evaluated in a total of 162 H&E stained sections.

There was no evidence of oncocytic cell development in the control group. In contrast, oncocytes were observed in 8 out of 13 NMO-treated rats: 2 in the adrenal sections (Fig. 1), 1 in the thyroid sections (Fig. 2), 3 in the pituitary sections (Fig. 3), and 2 in the pancreas sections (Fig. 4).

Interestingly, of these 8 animals, each one developed oncocytic transformation in only one organ. The percentages of the oncocytes in the sections are described in detail in Table 1. The cells in the testicle and bone sections were completely normal, with no oncocytic appearance.

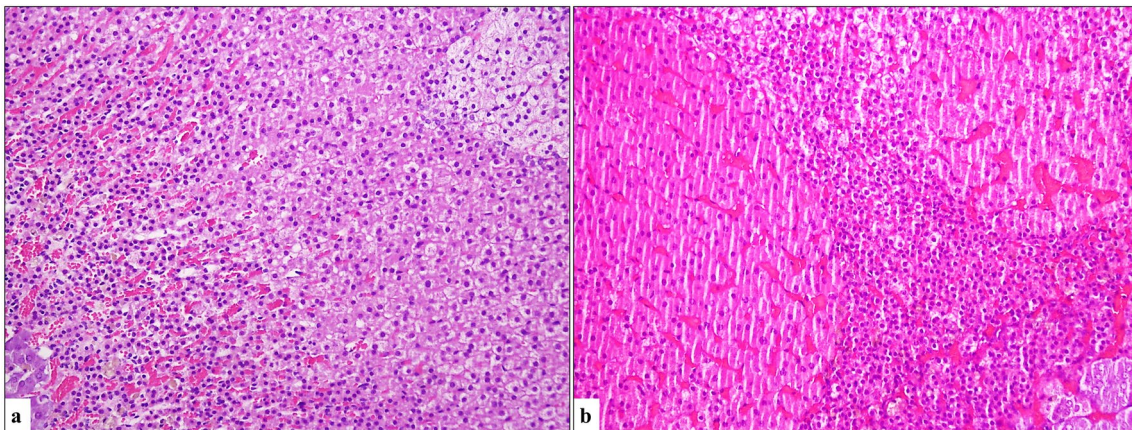


Fig. 1 **a** Normal adrenal gland layers (inner to outer layer) (H&E, $\times 200$). **b** Oncocytic cell groups with well-defined cell borders, deeply eosinophilic, granular cytoplasm, small round nuclei (H&E, $\times 200$)

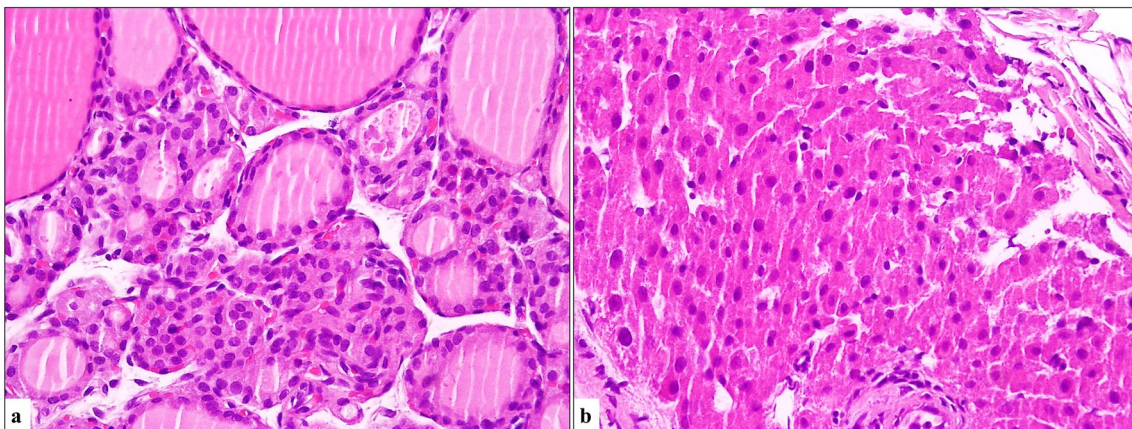


Fig. 2 **a** Thyroid parenchyma composed of normal follicle epithelial cells (H&E, $\times 400$). **b** Oncocytes with large size, prominent cell borders, eosinophilic, and granular cytoplasm on thyroid sections (H&E, $\times 400$)

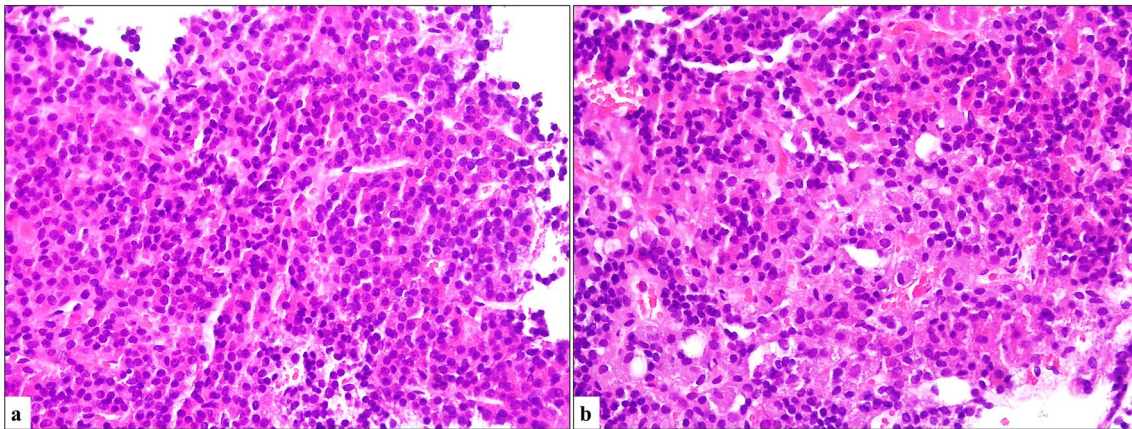


Fig. 3 **a** Normal pituitary tissue showing relative cellular monomorphism with a single cell type (H&E, ×400). **b** Oncocytic cells scattered in the pituitary parenchyma (H&E, ×400)

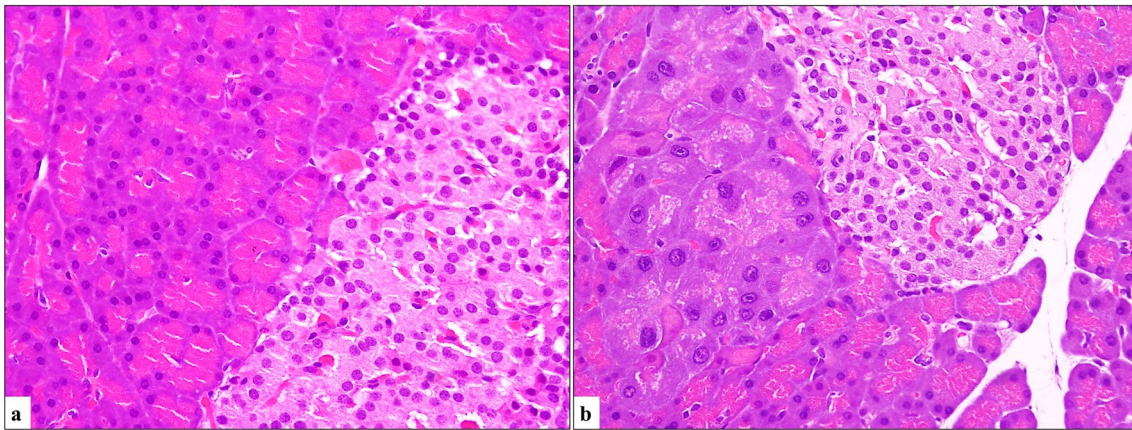


Fig. 4 **a** Normal pancreatic parenchyma (H&E, ×400). **b** Oncocytic cells located between acinar cells and endocrine cells (H&E, ×400)

Table 1 The percentages of oncocytic cells in the sections of the endocrine organs of the NMO-treated rats

Rat #	Percentages of oncocytes					
	Adrenal	Thyroid	Pituitary	Pancreas	Bone	Testis
1	10	–	–	–	–	–
2	10	–	–	–	–	–
3	–	–	–	–	–	–
4	–	–	–	–	–	–
5	–	–	10	–	–	–
6	–	–	20	–	–	–
7	–	–	–	–	–	–
8	–	–	–	5	–	–
9	–	–	–	5	–	–
10	–	20	–	–	–	–
11	–	–	10	–	–	–
12	–	–	–	–	–	–
13	–	–	–	–	–	–

Discussion

The results of the present study showed that NMO is a chemical compound which induces oncocytic transformation in the rat endocrine organs, i.e., adrenal, thyroid and pituitary glands, and pancreas. To date, in terms of oncocytic change, rat kidney has been the only proven organ which developed oncocytic tubules and oncocytoma after exposure to NMO in various animal studies. Similar to the methods of our study, Nogueira et al. administered single doses of 320 mg/kg of NMO by a gavage tube to a group of rats and started killing the rats 50 weeks after the start of the experiment [20]. Besides this group, three other groups of rats were given NMO in single doses of 200 mg per body weight dissolved in the drinking water at concentrations of 50 mg/100 ml or 20 mg/100 ml for 7 or 3 weeks. By the week 50 after the start of NMO administration, renal clear cell tumors and/or tubules were most frequently observed in the group which was treated with 320 mg of NMO per body weight and the authors reported that the granular acidophilic cells with larger central nuclei were more prevalent than clear cell lesions in all sections. Previous studies reported the appearance of oncocytic renal tubules after 15 and 23 weeks' exposure to NMO [15, 16]. In our study, we aimed to increase the likelihood of developing oncocytic lesions in the endocrine organs by maintaining the rats for 52 weeks.

The cytoplasm of an oncoocyte is described as "swollen" which is due to mainly the presence of numerous abnormal mitochondria in the cellular cytoplasm [22, 23]. The main mechanism for this granular, large, and eosinophilic cytoplasm filled with mitochondria has been indicated as the impairments in the mitochondrial DNA, which is supposed to be more susceptible than nuclear DNA to environmental carcinogens [12]. The accumulation of mitochondria has been attributed to the compensatory mechanism following exposure to toxic substances.

In our study, pituitary gland was clearly the most affected endocrine gland by the administration of NMO, in terms of oncocytic transformation. The well-vascularization of pituitary gland by the hypothalamohypophyseal portal system may be a possible explanation for this outcome, by making the gland tissue more sensitive to the environmental endocrine disruptors [24]. Oncocytic change has been mainly demonstrated in gonadotroph adenomas. A hundred cases with gonadotroph adenomas were analyzed by Young et al. and a patchy acidophilic appearance corresponding to oncocytic change was reported for most of the specimens [25]. In another study, oncocytic adenomas were described as clinically inactive pituitary adenomas, together with null cell adenomas and gonadotroph adenomas [26]. In short, the asymptomatic nature of oncocytic pituitary adenomas

may cause an underestimation of the actual prevalence of pituitary adenomas with oncocytic features, especially for the areas with elevated *N*-nitroso compounds in the air and/or drinking water.

Oncocytic transformation is not an uncommon pathological finding in both benign and malignant thyroid lesions. In a recent review, the oncocytic change in thyroid pathology has been discussed in detail and it has been described in thyroiditis, thyroid follicular adenomas, follicular-patterned differentiated thyroid carcinomas, papillary carcinomas, poorly differentiated carcinomas, and medullary thyroid carcinomas [13]. In line with the literature, our study demonstrated that NMO, a tumorigenic chemical, induced an oncocytic transformation in the thyroid tissue of one rat with a high percentage (20%) of oncoocytes.

In various reviews and case reports, oncocytic neoplasms have been described for both adrenal gland and pancreas [27–30]. Intraductal oncocytic papillary neoplasms (IOPNs), the precursor lesions of invasive carcinoma, are generally cystic neoplasms, which may cause pancreatic ductal obstruction. In contrast, oncocytic neoplasms arising in the adrenal glands are mostly benign and nonfunctional tumors, which rarely transform to oncocytic adrenocortical carcinoma. There are limited data on genetic mutations in IOPNs and adrenal oncocytic neoplasms. In the present study, oncoocytes were observed in the adrenal glands of two rats and in the pancreas tissues from another two rats with relatively low percentages of oncoocytes (5%) compared to the adrenal glands (10%). To date, there has been no report which described any oncocytic change in bone and testicular tissues. In parallel with the literature, we observed no oncocytic transformation in the sections of the bone and testicular tissues from the NMO-treated rats.

Our study has both a limitation and strength. The limitation was the lack of histopathological evaluation of those three rats' organs. Even though those three deaths were unexpected during the experiment, the histopathological evaluation could not be performed, because the rats were found dead during daily observation, but not at the time of death; thus, the organs were not suitable for any examination. A future experiment with a live monitoring which gives an online signal to the investigators in case of any abnormality in vital signs of the rats would give a more accurate data about the deceased rats during the study. The strength of our study is the absence of any confounding factors which might influence the observed oncocytic transformation. All rats were kept in the same standard laboratory environment and killed within the same daytime period in 2 consecutive days to eliminate the circadian rhythm differences between the groups at the time of sacrifice.

In conclusion, we showed that NMO induced an oncogenic change in pancreas, thyroid, pituitary, and adrenal glands. To the best of our knowledge, no identified specific environmental risk factors that lead to an oncogenic transformation in endocrine glands have been reported previously. One reason for the lack of extensive research in this area may be that oncogenic lesions are often characterized as benign tumors, which may not be a priority for researchers. However, a few oncogenic malignant tumors such as oncogenic adrenocortical carcinoma, oncogenic intraductal carcinoma of pancreas, oncogenic differentiated, poorly differentiated and medullary thyroid carcinomas, have also been reported. Therefore, given the increasing prevalence of endocrine-disrupting chemicals in the environment, personal care products, manufactured goods and food sources, there is a need to advance our understanding of the pathological mechanisms underlying oncogenesis in endocrine organs.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by CÖ, TŞ, BYB, and GD. The first draft of the manuscript was written by EG, MS, and DK and all authors commented on previous versions of the manuscript. BÇ, ZC, and AS critically revised, edited the article and served as scientific advisors. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval of this study was obtained from the Animal Experiments Local Ethics Committee of Kocaeli University (KOU HADYEK 2/2-2021, 25.02.2021). This study was complied with all national and local regulations as a minimum standard with regard to the care and use of animals within our establishments.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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References

- Zhao B, Nakada N, Okumura K, Zhou J, Tanaka H. *N*-nitrosomorpholine behavior in sewage treatment plants and urban rivers. *Water Res.* 2019;15:163.
- Spiegelhalter B, Preussmann R. Occupational nitrosamine exposure. 1. rubber and tyre industry. *Carcinogenesis.* 1983;4(9):1147–52.
- Spiegelhalter B, Preussmann R. Contamination of toiletries and cosmetic products with volatile and nonvolatile *N*-nitroso carcinogens. *J Cancer Res Clin Oncol.* 1984;108(1):160–3.
- Brunnemann KD, Hoffmann D. Assessment of the carcinogenic *N*-nitrosodiethanolamine in tobacco products and tobacco smoke. *Carcinogenesis.* 1981;2(11):1123–7.
- Jin SJ, Sang HL, Kap JJ, Choi YC, Woong YP, In HK, et al. Hepatotoxic *N*-nitrosomorpholine-induced carcinogenesis in rat liver: ex vivo exploration of preneoplastic and neoplastic hepatocytes. *Exp Mol Pathol.* 2003;74(1):74–83.
- Masui T, Nakanishi H, Inada KI, Imai T, Mizoguchi Y, Yada H, et al. Highly metastatic hepatocellular carcinomas induced in male F344 rats treated with *N*-nitrosomorpholine in combination with other hepatocarcinogens show a high incidence of p53 gene mutations along with altered mRNA expression of tumor-related genes. *Cancer Lett.* 1997;112(1):33–45.
- Klein RG, Spiegelhalter B, Preussmann R. Inhalation carcinogenesis of *N*-nitrosomorpholine (NMOR) in rats and hamsters. *Exp Pathol.* 1990;40(4):189–95.
- Mohr U, Reznik G, Reznik-Schüller H. Carcinogenic effects of *N*-nitrosomorpholine and *N*-nitrosopiperidine on European hamster (*Cricetus cricetus*). *J Natl Cancer Inst.* 1974;53(1):231–7.
- Reznik-Schüller H. Sequential morphologic alterations in the bronchial epithelium of Syrian golden hamsters during *N*-nitrosomorpholine-induced pulmonary tumorigenesis. *Am J Pathol.* 1977;89:59–66.
- Hecht SS, Lijinsky W, Kovatch RM, Chung FL, Saavedra JE. Comparative tumorigenicity of *N*-nitroso-2-hydroxymorpholine, *N*-nitrosodiethanolamine and *N*-nitrosomorpholine in A/J mice and F344 rats. *Carcinogenesis.* 1989;10(8):1475–7.
- Montone KT, Baloch ZW, LiVolsi VA. The thyroid Hürthle (oncogenic) cell and its associated pathologic conditions: a surgical pathology and cytopathology review. *Arch Pathol Lab Med.* 2008;132(8):1241–50.
- Máximo V, Soares P, Lima J, Cameselle-Teijeiro J, Sobrinho-Simões M. Mitochondrial DNA somatic mutations (point mutations and large deletions) and mitochondrial DNA variants in human thyroid pathology: a study with emphasis on Hürthle cell tumors. *Am J Pathol.* 2002;160(5):1857–65.
- Asa SL, Mete O. Oncogenic change in thyroid pathology. *Front Endocrinol (Lausanne).* 2021;3:12.
- Krech R, Zerban H, Bannasch P. Mitochondrial anomalies in renal oncocytes induced in rat by *N*-nitrosomorpholine. *Eur J Cell Biol.* 1981;25(2):331–9.
- Bannasch P, Krech R, Zerban H. Morphogenese und Mikromorphologie epithelialer Nierentumoren bei Nitrosomorpholin-vergifteten Ratten - III. Onkocytentubuli und Onkocytome. *Zeitschrift für Krebsforsch und Klin Onkol.* 1978;92(1):87–104.
- Ahn YS, Rempel A, Zerban H, Bannasch P. Over-expression of glucose transporter isoform GLUT1 and hexokinase I in rat renal oncocytic tubules and oncocytomas. *Virchows Arch.* 1994;425(1):63–8.
- Mayer D, Weber E, Kadenbach B, Bannasch P. Immunocytochemical demonstration of cytochrome c oxidase as a marker for renal oncocytes and oncocytomas. *Toxicol Pathol.* 1989;17:46–9.
- Tsuda H, Hacker HJ, Katayama H, Masui T, Ito N, Bannasch P. Correlative histochemical studies on preneoplastic and neoplastic lesions in the kidney of rats treated with nitrosamines. *Virchows Arch B Cell Pathol Incl Mol Pathol.* 1986;51(1):385–404.
- Nogueira E, Bannasch P. Cellular origin of rat renal oncocytoma. *Lab Invest.* 1988;59(3):337–43.
- Nogueira E, Klimek F, Weber E, Bannasch P. Collecting duct origin of rat renal clear cell tumors. *Virchows Arch B Cell Pathol Incl Mol Pathol.* 1989;57(1):275–83.
- Máximo V, Botelho T, Capela J, Soares P, Lima J, Taveira A, et al. Somatic and germline mutation in GRIM-19, a dual function gene involved in mitochondrial metabolism and cell death, is linked to mitochondrion-rich (Hürthle cell) tumours of the thyroid. *Br J Cancer.* 2005;92(10):1892–8.
- Satoh M, Yagawa K. Electron microscopic study on mitochondria in Hürthle cell adenoma of thyroid. *Pathol Int.* 1981;31(6):1079–87.
- Sobrinho-Simões M, Máximo V, De Castro IV, Fonseca E, Soares P, Garcia-Rostan G, et al. Hürthle (oncogenic) cell tumors of thyroid: etiopathogenesis, diagnosis and clinical significance. *Int J Surg Pathol.* 2005;13(1):29–35.
- Ganapathy MK, Tadi P. *Anatomy, Head and Neck, Pituitary Gland.* StatPearls [Internet]. 2022;
- Young WF, Scheithauer BW, Kovacs KT, Horvath E, Davis DH, Randall RV. Gonadotroph adenoma of the pituitary gland: a clinicopathologic analysis of 100 cases. *Mayo Clin Proc.* 1996;71(7):649–56.
- Saeger W. Hypophysentumoren. *Pathologie.* 2003;24(4):255–64.

27. Mearini L, Del Sordo R, Costantini E, Nunzi E, Porena M. Adrenal oncocytic neoplasm: a systematic review. *Urol Int*. 2013;91(2):125–33.
28. Virarkar M, Patnana M, Vikram R, Bhargava P, Birkenfeld E, Sagebiel T. Unravelling adrenal oncocytic neoplasm. *Clin Genitourin Cancer*. 2019;17(1):e216–20.
29. Munley J, Chang MD, Thomas RM. Intraductal oncocytic papillary neoplasm of the pancreas. *J Gastrointest Surg*. 2021;25(1):319–21.
30. Kallen ME, Naini BV. Intraductal oncocytic papillary neoplasms of the pancreas. *Arch Pathol Lab Med*. 2016;140(9):992–6.

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