Article

Inhibin Alpha Expression in Human Tumors: A Tissue Microarray Study on 12,212 Tumors

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Abstract: As a result of its expression in corresponding normal cell types, inhibin alpha (INHA) is used as an immunohistochemical marker for adrenocortical neoplasms and testicular or ovarian sex cord stromal tumors. However, other tumors can also express INHA. To comprehensively determine INHA expression in cancer, a tissue microarray containing 15,012 samples from 134 different tumor types and subtypes was analyzed by immunohistochemistry. INHA positivity was found in 72 of 134 tumor categories, including 26 categories with ≥1 strongly positive case. A moderate to strong INHA positivity was found in 100% of 37 granulosa cell tumors of the ovary, 100% of 43 other sex cord stromal tumors of the ovary/testis, 100% of 31 granular cell tumors, 78.5% of 28 adenomas, 44% of 25 carcinomas of the adrenal cortex, and 46.7% of 15 pancreatic acinar cell carcinomas. At least a weak INHA positivity was seen in <33% of cases of 46 additional tumor entities. In summary, these data support the use of INHA antibodies for detecting sex cord stromal tumors, granular cell tumors, and adrenocortical neoplasms. Since INHA can also be found in other tumor entities, INHA immunohistochemistry should only be considered as a part of any panel for the distinction of tumor entities.

Keywords: inhibin A (INHA); tissue micro array; immunohistochemistry; human tumors; cancer aggressiveness

1. Introduction

The inhibin alpha subunit protein (INHA) is a member of the TGF-beta (transforming growth factor-beta) superfamily encoded by a gene located at 2q35 [1–3]. It combines with the A and B type proteins of the inhibin beta subunits to form inhibin protein complexes that negatively regulate the secretion of follicle-stimulating hormone (FSH) from the pituitary gland [4–6]. Inhibin has also been suggested to inhibit gonadal stromal cell proliferation and to possess a tumor suppressive activity [4].

Among normal tissues, INHA staining is found in adrenocortical cells, Sertoli and Leydig cells of the testis, and the placenta [7]. Accordingly, inhibin alpha is currently used as an immunohistochemical marker for adrenocortical tumors and sex cord stromal tumors of the testis and the ovary [7–9]. However, a systematic analysis of inhibin alpha across human cancer types would be highly desirable to understand the diagnostic value of inhibin alpha detection. This is all the more pressing since other tumor entities have also been reported to express inhibin alpha across many tumor types, although the reported positivity rates are highly variable. For example, inhibin alpha positivity has been described in 41–100% of granulosa cell tumors of the ovary [10–12], 25–100% of adrenocortical...
carcinomas [13–16], 0–100% of mucinous carcinomas of the ovary [17–20], 0–63% of serous high-grade carcinomas of the ovary [12,21,22], 0–60% of Brenner tumors of the ovary [12,22,23], 0–75% of endometroid carcinomas of the ovary [12,18], and 0–16% of pheochromocytomas [14–16,24–29]. These conflicting data may be caused by the different antibodies, immunostaining protocols, and criteria used to determine INHA positivity in these studies.

To better understand the prevalence and significance of INHA expression in cancer, a comprehensive study analyzing a large number of neoplastic and non-neoplastic tissues under highly standardized conditions is needed. We therefore analyzed INHA expression in more than 15,000 tumor tissue samples from 134 different tumor types and subtypes, as well as 76 non-neoplastic tissue categories by immunohistochemistry (IHC) in a tissue microarray (TMA) format in this study.

2. Materials and Methods

2.1. Tissue Microarrays (TMAs)

Our normal tissue TMA was composed of 8 samples from 8 different donors for each of 76 different normal tissue types (608 samples on one slide). The cancer TMAs contained a total of 15,012 primary tumors from 134 tumor types and subtypes. The arrayed cancer samples were mainly of Caucasian origin and underwent surgery at the University Medical Center Hamburg-Eppendorf between 1992 and 2016. Detailed histopathological data were available for 2,351 colorectal adenocarcinomas, 192 neuroendocrine tumors, and 801 clear cell renal cell carcinomas. These tumors were distributed across 48 TMA blocks containing between 80 and 522 tissue spots with a diameter of 0.6 mm each. For a subset of 531 kidney cancer patients, clinical follow-up data were also accessible with a median follow-up time of 40 months (range 1–250). The composition of normal and cancer TMAs is described in the Results section. All samples were from the archives of the Institutes of Pathology, University Hospital of Hamburg, Germany, the Institute of Pathology, Clinical Center Osnabrueck, Germany, and the Department of Pathology, Academic Hospital Fuerth, Germany. Tissues were fixed in 4% buffered formalin and then embedded in paraffin. The TMA manufacturing process has been described earlier in detail [30–32]. In brief, one tissue spot (diameter: 0.6 mm) was transmitted from a tumor containing donor block in an empty recipient paraffin block. The use of archived remnants of diagnostic tissues for TMA manufacturing, their analysis for research purposes, and patient data were conducted according to local laws (HmbKHG, §12), and the analysis was approved by the local ethics committee (Ethics Commission Hamburg, WF-049/09). All work was carried out in compliance with the Helsinki Declaration.

2.2. Immunohistochemistry

Freshly cut TMA sections were immunostained on one day and in one experiment. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 min in an autoclave at 121 °C in a pH 7.8 buffer. A primary antibody specific for inhibin alpha (recombinant rabbit, MSVA-561R, MS Validated Antibodies, GmbH, Hamburg, Germany) was applied at 37 °C for 60 min at a dilution of 1:100. Bound antibody was then visualized using the EnVision Kit (Agilent, Santa Clara, CA, USA; #K5007) according to the manufacturer’s directions. For the purpose of antibody validation, the normal tissue TMA was also analyzed using a ready-to-use anti-inhibin diagnostic antibody (monoclonal mouse anti-human inhibin α, clone R1, Agilent, Santa Clara, CA, USA, cat.# IR058) according to the protocol suggested by the manufacturer. In brief, following pH 9 antigen retrieval, the TMA slide was stained in a DAKO auto-stainer Link48 with a FLEX detection system. An experienced pathologist performed manual analysis of the stained TMA slides. Hematoxylin- and eosin-stained sections were used for comparison in cases of questionable tumor cell content. For tumor tissues, the percentage of positive neoplastic cells was estimated, and the staining intensity was semi-quantitatively recorded (0, 1+, 2+, 3+). For statistical analyses, the staining results were categorized into four groups. Tumors without
any staining were considered negative. Tumors with 1+ staining intensity in \( \leq 70\% \) of tumor cells or 2+ intensity in \( \leq 30\% \) of tumor cells were considered weakly positive. Tumors with 1+ staining intensity in >70% of tumor cells, 2+ intensity in 31–70%, or 3+ intensity in \( \leq 30\% \) were considered moderately positive. Tumors with 2+ intensity in >70% or 3+ intensity in >30% of tumor cells were considered strongly positive.

2.3. Statistics
Statistical calculations were performed with JMP 14 software (SAS Institute Inc., Cary, NC, USA). Contingency tables and the chi\(^2\)-test were performed to search for associations between INHA and tumor phenotype. Survival curves were calculated according to Kaplan–Meier. The log-rank test was applied to detect significant differences between groups.

3. Results
3.1. Technical Issues
A total of 12,212 (81%) of 15,012 tumor samples were interpretable in our tumor TMA analysis. Non-interpretable samples demonstrated a lack of unequivocal tumor cells or loss of the tissue spot during technical procedures. A sufficient number of samples (<3) of each normal tissue type was evaluable.

3.2. Inhibin Alpha in Normal Tissues
By using MSVA-561R, strong INHA staining was found in Sertoli and Leydig cells of the testis, corpus luteum of the ovary, and the cyto- and syncytiotrophoblast, as well as chorion cells of the placenta (stronger staining in the first trimester than in mature placenta), and in adrenocortical cells. A more variable staining intensity ranging from weak to strong was seen in the amnion and decidua cells of the placenta, as well as in follicular, granulosa, and some stroma cells of the ovary. Scattered INHA-positive epithelial cells were also seen in the pancreas and the adenohypophysis. Representative images of INHA-positive normal tissues are shown in Figure 1. All these cell types also stained positive if the monoclonal mouse anti-human inhibin alpha antibody clone R1 was used (Figure S1). INHA staining was not seen in any other analyzed tissues, including skeletal muscle, heart muscle, smooth muscle, myometrium, fat, transitional mucosa of the anal canal, urothelium of the renal pelvis and urinary bladder, lymph node, spleen, thymus, tonsil, mucosa of the stomach, duodenum, ileum, appendix, colon, rectum and gallbladder, liver, parotid gland, submandibular gland, sublingual gland, Brunner’s gland of the duodenum, kidney, prostate, seminal vesicle, epididymis, bronchial glands, lung, breast, endocervix, endometrium, fallopian tube, thyroid, parathyroid gland, cerebellum, cerebrum, and the neurohypophysis.
Figure 1. Inhibin alpha (INHA) immunostaining in normal tissues. The panels show an INHA immunostaining of variable intensity in Sertoli and Leydig cells of the testis (A), corpus luteum of the ovary (B), cortical cells of the adrenal gland (C), cytotrophoblast cells of the first trimester placenta (D), and of the mature placenta (E), chorion cells of the placenta (F), decidua cells in placenta adjacent tissue (G), and granulosa cells of follicular cysts of the ovary (H). INHA staining is completely absent in the liver (I) and the fallopian tube (J).

3.3. Inhibin Alpha in Cancer

Positive INHA immunostaining was detectable in 583 (4.8%) of the 12,212 analyzable tumors, including 351 (2.9%) with weak, 66 (0.5%) with moderate, and 167 (1.4%) with strong INHA positivity. Overall, 72 (54%) of 134 tumor categories showed detectable INHA expression, with 26 (19%) tumor categories including at least one case with strong positivity (Table 1).

The highest rate of positive staining and the highest levels of expression were found in various types of sex cord stromal tumors of the testis and the ovary (100% positive), granular cell tumors (100%), granulosa cell tumors of the ovary (100%), and adrenal cortical adenomas (93%) and carcinomas (80%), as well as in acinar cell carcinomas of the pancreas (80%). Sixty additional tumor entities showed INHA immunostaining not only less frequently but also at lower intensity. Of note, tumor cell nests in the ovary were often surrounded by a conspicuous layer of INHA positive stromal cells. This was independent of the tumor type. A comparison with the histopathologic parameters of cancer aggressiveness and/or clinical data revealed significant associations between INHA positivity and nodal metastasis in colorectal adenocarcinoma ($p = 0.0494$) and high Thoenes' grade in clear cell renal cell carcinoma ($p = 0.0498$), as well as a tendency towards more nodal metastases in INHA-positive neuroendocrine tumors, although this relationship did not reach statistical significance ($p = 0.0824$; Table 2).
Table 1. Inhibin alpha (INHA) immunostaining in human tumors.

| Tumor Entity                                      | On TMA (n) | Analyzable (n) | Inhibin Alpha (INHA) IHC Result |
|-------------------------------------------------|------------|----------------|---------------------------------|
| Tumors of the skin                              |            |                |                                 |
| Pilomatrixoma                                    | 35         | 33             | 100.0                          |
| Basal cell carcinoma                             | 88         | 77             | 100.0                          |
| Benign nevus                                     | 29         | 23             | 100.0                          |
| Squamous cell carcinoma of the skin             | 90         | 83             | 100.0                          |
| Malignant melanoma                               | 46         | 41             | 100.0                          |
| Malignant melanoma Lymph node metastasis        | 86         | 83             | 100.0                          |
| Merkel cell carcinoma                            | 46         | 39             | 100.0                          |
| Squamous cell carcinoma of the larynx            | 109        | 101            | 95.0                           |
| Squamous cell carcinoma of the pharynx           | 60         | 60             | 100.0                          |
| Oral squamous cell carcinoma (floor of the mouth)| 130        | 128            | 98.4                           |
| Tumors of the head and neck                      |            |                |                                 |
| Pleomorphic adenoma of the parotid gland         | 50         | 44             | 100.0                          |
| Warthin tumor of the parotid gland               | 49         | 46             | 100.0                          |
| Basal cell adenoma of the salivary gland         | 15         | 14             | 100.0                          |
| Adenocarcinoma of the lung                       | 196        | 122            | 82.0                           |
| Squamous cell carcinoma of the lung              | 80         | 48             | 95.8                           |
| Small cell carcinoma of the lung                 | 16         | 13             | 92.3                           |
| Tumors of the lung, pleura, and thymus           |            |                |                                 |
| Mesothelioma, epithelioid                        | 39         | 23             | 91.3                           |
| Mesothelioma, other types                        | 76         | 53             | 98.1                           |
| Thymoma                                          | 29         | 27             | 100.0                          |
| Squamous cell carcinoma of the vagina            | 78         | 53             | 100.0                          |
| Squamous cell carcinoma of the vulva             | 130        | 123            | 99.2                           |
| Squamous cell carcinoma of the cervix            | 128        | 124            | 98.4                           |
| Adenocarcinoma of the cervix                     | 21         | 21             | 100.0                          |
| Endometrioid endometrial carcinoma               | 236        | 217            | 88.5                           |
| Endometrial serous carcinoma                     | 82         | 66             | 100.0                          |
| Carcinosarcoma of the uterus                     | 48         | 43             | 90.7                           |
| Endometrial carcinoma, high grade, G3            | 13         | 12             | 83.3                           |
| Endometrial clear cell carcinoma                 | 8          | 7              | 71.4                           |
| Tumors of the female genital tract               |            |                |                                 |
| Serous carcinoma of the ovary                    | 559        | 360            | 89.4                           |
| Mucinous carcinoma of the ovary                  | 96         | 68             | 97.1                           |
| Clear cell carcinoma of the ovary                | 50         | 39             | 92.3                           |
| Carcinosarcoma of the ovary                      | 47         | 37             | 89.2                           |
| Granulosa cell tumor of the ovary                | 37         | 36             | 0.0                            |
| Leydig cell tumor of the ovary                   | 4          | 4              | 0.0                            |
| Sertoli cell tumor of the ovary                  | 1          | 1              | 0.0                            |
| Sertoli Leydig cell tumor of the ovary           | 3          | 3              | 0.0                            |
| Steroid cell tumor of the ovary                  | 3          | 3              | 0.0                            |
| Brenner tumor                                    | 41         | 37             | 100.0                          |
| Invasive breast carcinoma of no special type     | 80         | 74             | 95.9                           |
| Tumors of the breast                             |            |                |                                 |
| Lobular carcinoma of the breast                  | 122        | 98             | 99.0                           |
| Medullary carcinoma of the breast                | 15         | 15             | 100.0                          |
| Tubular carcinoma of the breast                  | 18         | 15             | 100.0                          |
| Mucinous carcinoma of the breast                 | 22         | 15             | 100.0                          |
| Phyllodes tumor of the breast                    | 50         | 48             | 100.0                          |
| Adenomatous polyp, low-grade dysplasia           | 50         | 45             | 100.0                          |
| Adenomatous polyp, high-grade dysplasia          | 50         | 47             | 100.0                          |
| Tumors of the digestive system                   |            |                |                                 |
| Squamous cell carcinoma of the esophagus         | 76         | 71             | 97.2                           |
| Squamous cell carcinoma of the anal canal         | 89         | 79             | 98.7                           |
| Cholangiocarcinoma                               | 113        | 95             | 78.9                           |
| Hepatocellular carcinoma                        | 50         | 48             | 100.0                          |
| Ductal adenocarcinoma of the pancreas            | 612        | 322            | 97.5                           |
| Pancreatic/Ampullary adenocarcinoma              | 89         | 57             | 100.0                          |
| Acinar cell carcinoma of the pancreas            | 16         | 15             | 20.0                           |
| Gastrointestinal stromal tumor (GIST)            | 50         | 47             | 100.0                          |
| Table 1. Cont. |
|----------------|
| Tumor Entity | On TMA (n) | Analyzable (n) | Inhibin Alpha (INHA) IHC Result |  |
|               |            | (n) | | Negative (%) | Weak (%) | Moderate (%) | Strong (%) |  |
| **Tumors of the urinary system** | | | | | | | | |
| Non-invasive papillary urothelial carcinoma, pTa G2 low grade | 177 | 133 | 100.0 | 0.0 | 0.0 | 0.0 |
| Non-invasive papillary urothelial carcinoma, pTa G2 high grade | 141 | 106 | 99.1 | 0.9 | 0.0 | 0.0 |
| Non-invasive papillary urothelial carcinoma, pTa G3 | 219 | 163 | 99.4 | 0.6 | 0.0 | 0.0 |
| Urothelial carcinoma, pT2-4 G3 | 1318 | 1047 | 97.0 | 2.7 | 0.1 | 0.2 |
| Squamous cell carcinoma of the bladder | 22 | 21 | 100.0 | 0.0 | 0.0 | 0.0 |
| Small cell neuroendocrine carcinoma of the bladder | 23 | 22 | 95.5 | 4.5 | 0.0 | 0.0 |
| Sarcomatoid urothelial carcinoma | 25 | 11 | 100.0 | 0.0 | 0.0 | 0.0 |
| Urothelial carcinoma of the kidney pelvis | 62 | 62 | 98.4 | 1.6 | 0.0 | 0.0 |
| Clear cell renal cell carcinoma | 857 | 770 | 97.7 | 1.2 | 0.1 | 1.0 |
| Papillary renal cell carcinoma | 255 | 223 | 99.6 | 0.4 | 0.0 | 0.0 |
| Clear cell (tubulo) papillary renal cell carcinoma | 21 | 20 | 70.0 | 5.0 | 5.0 | 20.0 |
| **Tumors of the male genital organs** | | | | | | | | |
| Adenocarcinoma of the prostate, Gleason 3+3 | 83 | 83 | 100.0 | 0.0 | 0.0 | 0.0 |
| Adenocarcinoma of the prostate, Gleason 4+4 | 80 | 79 | 100.0 | 0.0 | 0.0 | 0.0 |
| Adenocarcinoma of the prostate, Gleason 5+5 | 85 | 85 | 100.0 | 0.0 | 0.0 | 0.0 |
| Adenocarcinoma of the prostate (recurrence) | 258 | 217 | 99.1 | 0.9 | 0.0 | 0.0 |
| Small cell neuroendocrine carcinoma of the prostate | 19 | 17 | 100.0 | 0.0 | 0.0 | 0.0 |
| Seminoma | 621 | 591 | 98.0 | 1.7 | 0.2 | 0.2 |
| Embryonal carcinoma of the testis | 50 | 22 | 100.0 | 0.0 | 0.0 | 0.0 |
| Leydig cell tumor of the testis | 30 | 30 | 0.0 | 0.0 | 0.0 | 100.0 |
| Sertoli cell tumor of the testis | 2 | 2 | 0.0 | 0.0 | 0.0 | 50.0 |
| Sex cord stromal tumor of the testis | 1 | 1 | 0.0 | 0.0 | 0.0 | 100.0 |
| Spermatocytic tumor of the testis | 50 | 25 | 100.0 | 0.0 | 0.0 | 0.0 |
| Teratoma | 50 | 34 | 91.2 | 8.8 | 0.0 | 0.0 |
| Squamous cell carcinoma of the penis | 80 | 80 | 98.8 | 0.0 | 1.3 | 0.0 |
| Adenoma of the thyroid gland | 113 | 99 | 99.0 | 1.0 | 0.0 | 0.0 |
| Papillary thyroid carcinoma | 391 | 250 | 84.4 | 13.2 | 2.4 | 0.0 |
| Follicular thyroid carcinoma | 154 | 109 | 97.2 | 2.8 | 0.0 | 0.0 |
| Medullary thyroid carcinoma | 111 | 94 | 94.7 | 5.3 | 0.0 | 0.0 |
| Parathyroid gland adenoma | 43 | 42 | 100.0 | 0.0 | 0.0 | 0.0 |
| Anaplastic thyroid carcinoma | 45 | 43 | 95.3 | 4.7 | 0.0 | 0.0 |
| **Tumors of endocrine organs** | | | | | | | | |
| Adrenal cortical adenoma | 50 | 28 | 7.1 | 14.3 | 32.1 | 46.4 |
| Adrenal cortical carcinoma | 26 | 25 | 20.0 | 36.0 | 20.0 | 24.0 |
| Phaeochromocytoma | 50 | 49 | 93.9 | 4.1 | 2.0 | 0.0 |
| Appendix, neuroendocrine tumor (NET) | 22 | 13 | 84.6 | 15.4 | 0.0 | 0.0 |
| Colorectal, neuroendocrine tumor (NET) | 12 | 8 | 75.0 | 25.0 | 0.0 | 0.0 |
| Ileum, neuroendocrine tumor (NET) | 49 | 42 | 92.9 | 7.1 | 0.0 | 0.0 |
| Lung, neuroendocrine tumor (NET) | 19 | 17 | 100.0 | 0.0 | 0.0 | 0.0 |
| Pancreas, neuroendocrine tumor (NET) | 97 | 84 | 79.8 | 8.3 | 2.4 | 9.5 |
| Colorectal, neuroendocrine carcinoma (NEC) | 12 | 7 | 100.0 | 0.0 | 0.0 | 0.0 |
| Gallbladder, neuroendocrine carcinoma (NEC) | 4 | 3 | 66.7 | 33.3 | 0.0 | 0.0 |
| Pancreas, neuroendocrine carcinoma (NEC) | 14 | 14 | 85.7 | 14.3 | 0.0 | 0.0 |
| Hodgkin Lymphoma | 103 | 95 | 100.0 | 0.0 | 0.0 | 0.0 |
| Small lymphocytic lymphoma, B-cell type (B-SLL/B-CLL) | 50 | 50 | 100.0 | 0.0 | 0.0 | 0.0 |
| Diffuse large B cell lymphoma (DLBCL) | 113 | 113 | 99.1 | 0.0 | 0.0 | 0.9 |
| Follicular lymphoma | 88 | 87 | 100.0 | 0.0 | 0.0 | 0.0 |
| T-cell Non Hodgkin lymphoma | 25 | 25 | 96.0 | 0.0 | 0.0 | 4.0 |
| Mantle cell lymphoma | 18 | 18 | 100.0 | 0.0 | 0.0 | 0.0 |
| Marginal zone lymphoma | 16 | 15 | 100.0 | 0.0 | 0.0 | 0.0 |
Table 1. Cont.

| Tumor Entity | On TMA (n) | Analyzable (n) | Inhibin Alpha (INHA) IHC Result |
|--------------|-----------|----------------|---------------------------------|
|              |           |                | Negative (%) | Weak (%) | Moderate (%) | Strong (%) |
| Diffuse large B-cell lymphoma (DLBCL) in the testis | 16 | 16 | 100.0 | 0.0 | 0.0 | 0.0 |
| Burkitt lymphoma | 5 | 2 | 100.0 | 0.0 | 0.0 | 0.0 |
| Tendosynovial giant cell tumor | 45 | 37 | 100.0 | 0.0 | 0.0 | 0.0 |
| Granular cell tumor | 53 | 31 | 0.0 | 0.0 | 12.9 | 87.1 |
| Leiomyoma | 50 | 48 | 100.0 | 0.0 | 0.0 | 0.0 |
| Leiomyosarcoma | 87 | 79 | 94.9 | 3.8 | 1.3 | 0.0 |
| Liposarcoma | 132 | 107 | 100.0 | 0.0 | 0.0 | 0.0 |
| Malignant peripheral nerve sheath tumor (MPNST) | 13 | 11 | 100.0 | 0.0 | 0.0 | 0.0 |

Tumors of soft tissue and bone

| Tumor Entity | On TMA (n) | Analyzable (n) | Inhibin Alpha (INHA) IHC Result |
|--------------|-----------|----------------|---------------------------------|
|              |           |                | Negative (%) | Weak (%) | Moderate (%) | Strong (%) |
| Myofibrosarcoma | 26 | 26 | 100.0 | 0.0 | 0.0 | 0.0 |
| Angiosarcoma | 73 | 55 | 72.7 | 14.5 | 12.7 | 0.0 |
| Angiomyolipoma | 91 | 65 | 100.0 | 0.0 | 0.0 | 0.0 |
| Dermatofibrosarcoma protuberans | 21 | 14 | 100.0 | 0.0 | 0.0 | 0.0 |
| Ganglieneuroma | 14 | 14 | 100.0 | 0.0 | 0.0 | 0.0 |
| Neurofibroma | 117 | 111 | 100.0 | 0.0 | 0.0 | 0.0 |
| Sarcoma, not otherwise specified (NOS) | 74 | 66 | 98.5 | 0.0 | 0.0 | 1.5 |
| Paragangioma | 41 | 41 | 92.7 | 2.4 | 2.4 | 2.4 |
| Ewing sarcoma | 23 | 13 | 100.0 | 0.0 | 0.0 | 0.0 |
| Rhabdomyosarcoma | 6 | 5 | 100.0 | 0.0 | 0.0 | 0.0 |
| Schwannoma | 121 | 115 | 97.4 | 2.6 | 0.0 | 0.0 |
| Synovial sarcoma | 12 | 8 | 75.0 | 12.5 | 12.5 | 0.0 |
| Osteosarcoma | 43 | 28 | 100.0 | 0.0 | 0.0 | 0.0 |
| Chondrosarcoma | 38 | 15 | 100.0 | 0.0 | 0.0 | 0.0 |
| Rhabdoid tumor | 5 | 5 | 100.0 | 0.0 | 0.0 | 0.0 |

Representative images of INHA positive tumors are shown in Figure 2.

Figure 2. INHA immunostaining in cancer. The panels show a cytoplasmatic INHA immunostaining of variable intensity in samples from a granulosa cell tumor of the ovary (A), a Leydig cell tumor (B) and Sertoli cell tumor of the testis (C), an adrenocortical carcinoma (D), a granular cell tumor from the floor of mouth (E), and a clear cell carcinoma of the kidney (F). INHA immunostaining is absent, however, in a testicular seminoma (G) and in tumor cells of a high-grade serous carcinoma of the ovary which contains INHA-positive stroma cells (H).
Table 2. INHA immunostaining and cancer phenotype.

| Colon adenocarcinoma | Primary tumor | pT1 | pT2 | pT3 | pT4 | n | Negative (%) | Weak (%) | Moderate (%) | Strong (%) | p       |
|----------------------|--------------|-----|-----|-----|-----|---|--------------|----------|-------------|-----------|--------|
|                      |              | 69  | 372 | 1042| 363 |   | 100.0       | 98.7     | 96.4        | 96.4      | 0.2243 |
| Regional lymph nodes | pN0          | 982 |     |     |     |   | 97.9        | 95.9     | 96.4        | 96.4      | 0.0494 |
|                      | pN+          | 860 |     |     |     |   | 97.2        | 97.2     | 97.2        | 97.2      | 0.0291 |
| Tumor localization   | left colon   | 930 |     |     |     |   | 97.2        | 97.7     | 97.7        | 97.7      | 0.8868 |
|                      | right colon  | 385 |     |     |     |   | 97.7        | 97.0     | 97.0        | 97.0      | 0.9188 |
| MMR status           | defective    | 71  |     |     |     |   | 97.2        | 97.2     | 97.2        | 97.2      | 0.0517 |
|                      | proficient   | 907 |     |     |     |   | 97.5        | 97.5     | 97.5        | 97.5      | 0.0517 |
| RAS mutation status  | mutated      | 226 |     |     |     |   | 94.7        | 94.7     | 94.7        | 94.7      | 0.0517 |
|                      | wildtype     | 292 |     |     |     |   | 97.6        | 97.6     | 97.6        | 97.6      | 0.0517 |
| BRAF mutation status | mutated      | 10  |     |     |     |   | 100.0       | 97.8     | 97.8        | 97.8      | 0.0517 |
|                      | wildtype     | 89  |     |     |     |   | 97.8        | 97.8     | 97.8        | 97.8      | 0.0517 |

| Clear cell renal cell carcinomas | ISUP grade | 1  | 235 | 96.6 | 0.0 | 0.0 | 0.4 | 0.0801  |
|                                 | 2           | 231 | 96.1 | 2.2 | 0.0 | 1.7 |     |
|                                 | 3           | 207 | 97.6 | 1.4 | 0.5 | 0.5 |     |
|                                 | 4           | 43  | 95.3 | 4.7 | 0.0 |     |     |
|                                 | Fuhrmann grade | 1  | 42  | 100.0 | 0.0 | 0.0 | 0.0 | 0.2987  |
|                                 | 2           | 422 | 97.6 | 1.2 | 0.0 | 1.2 |     |
|                                 | 3           | 209 | 98.1 | 1.0 | 0.5 | 0.5 |     |
|                                 | 4           | 52  | 94.2 | 5.8 | 0.0 |     |     |
|                                 | Thoenes’ grade | 1  | 267 | 99.3 | 0.7 | 0.0 | 0.0 | 0.0498  |
|                                 | 2           | 390 | 96.9 | 1.3 | 0.3 | 1.5 |     |
|                                 | 3           | 68  | 95.6 | 4.4 | 0.0 |     |     |
|                                 | UICC stage | 1  | 339 | 98.2 | 0.9 | 0.0 | 0.9 | 0.2994  |
|                                 | 2           | 37  | 100.0 | 0.0 | 0.0 |     |     |
|                                 | 3           | 91  | 97.8 | 1.1 | 1.1 | 0.0 |     |
|                                 | 4           | 74  | 95.9 | 4.1 | 0.0 |     |     |
|                                 | Primary tumor | 1  | 438 | 97.9 | 1.1 | 0.0 | 0.9 | 0.3615  |
|                                 | 2           | 73  | 98.6 | 0.0 | 0.0 | 1.4 |     |
|                                 | Regional lymph nodes | 0  | 122 | 96.7 | 0.8 | 0.8 | 1.6 | 0.4863  |
|                                 | ≥1           | 18  | 94.4 | 5.6 | 0.0 |     |     |
|                                 | Distant metastasis | 0  | 108 | 98.1 | 0.9 | 0.0 | 0.9 | 0.225   |
|                                 | ≥1           | 75  | 96.0 | 4.0 | 0.0 |     |     |

| Neuroendocrine tumors | Primary tumor | pT1 | pT2 | pT3 | pT4 | n | Negative (%) | Weak (%) | Moderate (%) | Strong (%) | p       |
|                       |              | 24  | 25  | 37  | 28  |   | 91.7        | 88.0     | 78.4        | 92.9      | 0.3256 |
|                       |              | 81  | 80  | 81  | 71  |   | 88.0        | 88.0     | 88.0        | 88.0      | 4.00   |
|                       |              | 4.0 | 4.0 | 4.0 | 4.0 |   | 88.0        | 88.0     | 88.0        | 88.0      | 4.00   |
|                       |              | 4.0 | 4.0 | 4.0 | 4.0 |   | 88.0        | 88.0     | 88.0        | 88.0      | 4.00   |
|                       |              | 4.0 | 4.0 | 4.0 | 4.0 |   | 88.0        | 88.0     | 88.0        | 88.0      | 4.00   |
|                       |              | 4.0 | 4.0 | 4.0 | 4.0 |   | 88.0        | 88.0     | 88.0        | 88.0      | 4.00   |
|                       |              | 4.0 | 4.0 | 4.0 | 4.0 |   | 88.0        | 88.0     | 88.0        | 88.0      | 4.00   |
|                       |              | 4.0 | 4.0 | 4.0 | 4.0 |   | 88.0        | 88.0     | 88.0        | 88.0      | 4.00   |
|                       | Regional lymph nodes | pN0 | 41  | 92.7 | 7.3 | 0.0 | 0.0 | 0.0824 |
|                       | pN+          | 58  | 82.8 | 6.9 | 3.4 | 6.9 |     |

4. Discussion

More than 12,000 tumors were successfully analyzed in this study. Considering the large scale of our study, our assay was extensively validated by comparing our IHC findings in normal tissues with RNA data derived from three different publicly accessible databases [33–36] and immunostaining data obtained by a second independent anti-INHA antibody. This approach has been suggested by the international working group for antibody validation (IWGAV) for the validation of IHC assays designed for formalin-fixed tissues [33]. To ensure as broad as possible a range of proteins to be tested for possible cross-reactivity, 76 different normal tissue categories were included in this analysis. The fact that INHA immunostaining was only seen in the testis, ovary, placenta, adrenal glands, pancreas, and the adenohypophysis supports the validity of our assay because INHA RNA expression was also detected in these organs. Additional validation comes from the staining of identical cell types such as the Sertoli and Leydig cells of the testis, corpus luteum, follicular, granulosa, and stroma cells of the ovary, cyto- and syncytiotrophoblast, as well
as chorion cells, amnion, and decidua cells of the placenta, and adrenocortical cells, as well as scattered epithelial cells in the pancreas by an independent second antibody (Figure S1).

INHA protein expression is generally considered an important diagnostic feature for adrenocortical tumors and granular cell tumors, as well as sex cord stromal tumors of the testis and the ovary [28,37–39]. The fact that the vast majority of these tumors showed a strong INHA expression in our study is thus consistent with the literature and confirms the utility of INHA immunostaining for supporting these diagnoses [40]. The extended analysis of 134 different tumor entities for INHA expression, including more than 80 tumor types and subtypes that had not been examined thus far for INHA expression showed, however, that INHA expression can occur in a much broader spectrum of tumors.

A small fraction of tumors of various categories showed a strong INHA positivity that was comparable to the expression levels of adrenocortical, granular cell, and sex cord stromal tumors. These especially included multiple cases of acinar cell carcinoma of the pancreas. This tumor entity had not been analyzed thus far for INHA expression. It constitutes a rare but highly malignant tumor derived from pancreatic acinar cells, a cell type showing low level INHA protein expression in our normal tissue screening. Other tumor entities which can show high-level INHA expression include, for example, neuroendocrine tumors of the pancreas, cholangiocarcinoma, adenocarcinoma of the lung, gastric adenocarcinoma, and clear cell renal cell carcinoma. The ability of renal carcinomas to highly express INHA is of particular relevance because INHA immunohistochemistry is used as a tool to distinguish normal or neoplastic adrenal tissue from clear cell renal cell carcinomas, as these entities may be difficult to distinguish by morphology alone [41–43]. Our data suggest that this clinically important distinction should not be solely based on the identification of high-level INHA expression in a tissue in question.

The majority of our INHA-positive cases showed INHA immunostaining in only a small fraction of tumor cells, often in the range of 1–10% of tumor cells, while adrenocortical, granular cell, and sex cord stromal tumors usually showed a moderate to strong INHA positivity in all or almost all tumor cells. These findings demonstrate that a focal weak to moderate INHA immunostaining should not be diagnostically overinterpreted. The biological role of focal low-level or even diffuse high-level INHA expression in cancers derived from cells that normally do not express INHA is unknown. The observation would, however, be consistent with a paracrine role of INHA in these tumors. INHA has recently been suggested as a novel paracrine factor for tumor angiogenesis and metastasis based on in vitro experiments demonstrating that tumor-cell-derived INHA can induce the growth of cultured endothelial cells through a signaling pathway involving the TGF beta co-receptor endoglin and its downstream activators of angiogenesis, ALK1 and SMAD1/5 [44]. The authors also show RNA data indicating a poor clinical outcome of INHA-positive tumors in ovarian cancers and renal cell carcinomas [44].

Due to the rarity of immunohistochemically detectable INHA expression in most cancer types, we were only able to compare INHA immunostaining data with available clinical data in neuroendocrine tumors, clear cell renal cell carcinoma, and colorectal adenocarcinoma. The fact that positive INHA immunostaining was marginally related to features of cancer aggressiveness in all these cancer types would be consistent with the notion that the neo-expression of INHA in cancers could exert a tumor-promoting effect, potentially through a paracrine activity of secreted INHA. A strong expression of INHA in tumor adjacent stroma cells observed in a fraction of otherwise INHA-negative ovarian carcinomas would also be consistent with the paracrine stimulation of tumor cell growth.

Our data provide a comprehensive ranking list of tumors according to their INHA expression across a large variety of tumor entities. It is almost certain that the use of different protocols, antibodies, interpretation criteria, and thresholds to define “positivity” have jointly caused the high diversity of literature data on INHA expression in tumors (summarized in Figure 3).
Our data corroborate that INHA is commonly expressed in various types of sex cord stromal tumors and granular cell tumors, as well as adrenal cortical neoplasms. Considering the fact that INHA expression can also be found in 60 other tumor entities, including 15 entities with a fraction of strongly positive cancers, INHA immunohistochemistry should only be applied as a part of a panel for the distinction of tumor entities. While the data from ourselves and others suggest a potential link between INHA expression and increased diversity in cancer cell biology, the clinical significance of specific INHA positivity patterns requires further investigation.

### Figure 3. INHA data from previous literature. The colors of the triangles represent the numbers of analyzed tumors in these studies: red: n = 1–9, yellow: n = 10–50, green: n >50. + indicates results of INHA data from previous literature.

The positivity rates described in the present study are thus specific to the reagents and protocols used in our laboratory. In contrast to previous studies using other reagents, relevant INHA1 immunostaining was not observed in adenocarcinomas of the esophagus or urothelial carcinomas, nor in mucinous, serous, or endometroid ovarian carcinomas. It is expected that different experimental conditions could change the INHA positivity rates—especially in tumors with low expression levels—but this would have little impact on the tumor ranking based on the INHA positivity rates.

### 5. Conclusions

Our data corroborate that INHA is commonly expressed in various types of sex cord stromal tumors and granular cell tumors, as well as adrenal cortical neoplasms. Considering the fact that INHA expression can also be found in 60 other tumor entities, including 15 entities with a fraction of strongly positive cancers, INHA immunohistochemistry should only be applied as a part of a panel for the distinction of tumor entities. While the data from ourselves and others suggest a potential link between INHA expression and increased diversity in cancer cell biology, the clinical significance of specific INHA positivity patterns requires further investigation.
aggressiveness in various cancer types, the functional role of INHA in these tumors awaits further investigation.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomedicines10102507/s1, Figure S1: IHC validation by comparison of antibodies. The panels show a concordance of immunostaining results obtained by two independent INHA antibodies. Using MSVA-561R, significant cytoplasmic staining is seen in adrenocortical cells (A), Sertoli and Leydig cells of the testis (B), the corpus luteum (C) and theca cells (D) of the ovary, decidua cells in the pregnant uterus (E), and in trophoblast cells of the first trimester placenta (F). Using anti-inhibin α (clone R1), comparable staining is seen in the adrenal gland (G), testis (H), corpus luteum (I) and theca cells (K) of the ovary, decidua cells (L), and in the placenta (M). The images A–F and G–M are from consecutive tissue sections. Table S1: List of the references and raw data used to create Figure 3.

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**Data Availability Statement:** All data generated or analyzed during this study are included in this published article.

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