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ORIGINAL ARTICLE

Metastatic Spread from Abdominal Tumor Cells to Parathymic Lymph Nodes

Gábor Király[1,2] · Zoltán Hargitai[3] · Ilona Kovács[3] · Gábor Szemán-Nagy[1] · István Juhász[2,4] · Gáspár Bánfalvi[1]

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Abstract

Metastatic studies on rats showed that after subrenal implantation of tumor cells under the capsule of the kidney or subhepatic implantation under Glisson's capsule of the liver generated primary tumors in these organs. It was assumed that tumor cells that escaped through the disrupted peripheral blood vessels of primary tumors entered the peritoneal cavity, crossed the diaphragm, and appeared in the thoracal, primarily in the parathymic lymph nodes. This explanation did not answer the question whether distant lymph nodes were reached via the blood stream from the primary tumor or through the thoracal lymphatic vessels. In this work, we investigated the metastatic pathway in C3H/HeJ mice, after direct intraperitoneal administration of murine SCC VII cells bypassing the hematogenic spread of tumor cells. The direct pathway was also mimicked by intraperitoneal injection of Pelican Ink colloidal particles, which appeared in the parathymic lymph nodes, similarly to the tumor cells that caused metastasis in the parathymic lymph nodes and in the thymic tissue. The murine peritoneal-parathymic lymph node route indicates a general mechanism of tumor progression from the abdominal effusion. This pathway starts with the growth of abdominal tumors, continues as thoracal metastasis in parathymic lymph nodes and may proceed as mammary lymph node metastasis.

Keywords Carcinoma cell line · Murine metastasis model · Parathymic lymph node · Colloidal ink

Introduction

In the development of drug and cancer therapy animal models, including the most economical rodent models are indispensable. Among the many ways of inducing tumors or metastases one possible way is the use of carcinogens (e.g. N-nitrosodimethylamine, 7,12-Dimethylbenz[a]anthracene) [1]. In other applications, tumor cell suspensions or tumor slices are implanted to follow tumorigenesis [2]. There are two major types of animal models to investigate tumor progression: *i)* the orthotopic tumor cell injection applies tumor cells identical with the host tissue, *ii)* in the ectopic model, the tumor cells are implanted subcutaneously [3]. The ectopic murine cancer model is often criticized for not representing the tumor progression reliable because the microenvironment plays a key role in the tumor formation [4]. The orthotopic implantation may result in metastasis in mice, while the ectopic method led exclusively to local tumors without metastasis [5].

Previous studies showed, that the orthotopic implantation of hepatocarcinoma or nephroblastoma rat tumor cells under the capsule turned out to be a reliable metastatic model [6]. The lymphatic drainage in the peritoneal cavity after intraperitoneal (*i.p.*) or retroperitoneal (*r.p.*) inoculation of bacteria (*Listeria monocytogenes*), similarly to the drainage of India ink, indicated that both agents were transported by the intraperitoneal macrophages to the thoracic lymph centre (*Lymphocentrum thoracicum ventrale*) and to the lymph nodes of the mediastinal lymph centre (*Lymphocentrum mediastinale*) [7]. Peritoneal cells migrated through the diaphragmatic "stomata" to the parathymic lymph glands via the mediastinal lymphatics [8]. Intraperitoneal injection of viral

István Juhász and Gáspár Bánfalvi contributed equally to this work.

✉ István Juhász
ji@med.unideb.hu

✉ Gáspár Bánfalvi
gaspar.banfalvi@gmail.com

[1] Department of Biotechnology and Microbiology, University of Debrecen, 1 Egyetem Square, Debrecen 4010, Hungary

[2] Department of Surgery and Operative Techniques, University of Debrecen, 98 Nagyerdei körút, Debrecen 4012, Hungary

[3] Department of Pathology, Kenézy Hospital, University of Debrecen, 2-28 Bartok Street, Debrecen 4031, Hungary

[4] Department of Dermatology, University of Debrecen, 98 Nagyerdei körút, Debrecen 4012, Hungary

particles, such as porcine circovirus type 2, were phagocytosed by intraperitoneal macrophages and transported toward the sternal lymph nodes of the ventral thoracic lymph centre [9]. Once they reached the tissues only particles with sizes less than a few hundred nanometers can permeate the leaky tumor vasculature. Such nano-sized particles of India ink can efficiently permeate out of the blood circulation into tumor tissues [10].

The administration of an exact number of tumor cells made it possible to follow the temporal aspects of tumor development in mice. During intraperitoneal administration, the tumor cells were injected into the abdominal cavity, where the tumor cells could adhere to peritoneal and retroperitoneal organs such as liver, spleen, kidney, and intestines. Some of the tumor cells could traverse the diaphragm through the stomata and appear in the thymus and lymph nodes of the mediastinum. Parathymic lymph nodes (PTNs) as the sentinel lymph nodes of the thoracal lymph node chain in mice were expected to accumulate tumor cells similarly to PTNs in rats [8]. It was reported that murine PTNs, unlike rat parathymic lymph nodes, cannot be removed from the thymus being harbored inside the thymus sack [11]. Upon implantation of tumor cells under the capsule of kidney or Glisson's capsule of the rat liver, some of the tumor cells were released through the disrupted peripheral blood vessels from the periphery of the primary tumors crossed the diaphragm, invaded the thoracal lymph node and accumulated in PTNs [8]. Once the immunogenic capacity of the last defence line of PTNs was exhausted, the metastatic tumor growth at the peripheral parts of PTNs was subjected to hypoxic angiogenesis and tissue disruptions. In agreement with the observations of others the presence of lymph node metastases signifies further metastatic spread and poor patient survival [12]. Cells contained in the efferent lymph were predominantly lymphocytes with a smaller population of macrophages [13]. Tumor cells that reached the PTNs, located in the upper cavity of the thorax could have invaded the lower part of the thoracal cavity, spread to other tissues whereas the main thoracic duct (*Ductus thoracicus*) carried the lymph to the blood after entering the subclavian vein. An expected outcome of this process could be the redistribution of tumor cells to other thoracal lymph nodes and other body parts.

This study summarizes the spread of cells released from abdominal tumors of rats and addresses questions related to the peritoneal, retroperitoneal and thoracal metastatic development by the intraperitoneal administration of murine tumor cells. Other small particles (Pelikan Ink) were also administered *i.p.* as their spread was expected to follow the same route from the abdomen to the thoracal cavity and to thoracal lymph nodes. One of the questions to be answered was the fate of abdominal tumor cells released into the abdomen. Direct administration of tumor cells into the abdomen and their appearance in PTNs intended to exclude the possibility of the spread of metastasis via the blood stream. In the rat model tumor cells were released from the abdominal tumors of the kidney or the liver, but the vast majority of the tumor cells proliferated in the primary tumor and could have circulated by the blood stream, filtered out and deposited in PTNs. The second question concerned the intra- or extrathymic location of parathymic nodes in mice. The appearance of SCC VII carcinoma cells in PTNs was tested by immunohistochemistry after preparing tissue sections from PTN tumors. Carcinoma metastasis in PTNs, expressing cytokeratin surface tumor markers was confirmed by immunohistochemical identification [11].

Materials and Methods

Murine Meatastsis Model and its Human Aspects

A syngenic murine tumor model with the implantation of human cells was used to decide whether or not tumor cells spread from the peritoneal cavity to thoracal thymic lymph nodes better known in mammals as internal mammary lymph nodes. These experiments were performed after metastatic studies related to thoracal, particularly parathymic lymph nodes were reported in rats after orthotopic implantation of different types of tumor cells [14, 15]. Rat PTNs are located outside the thymus capsule and separable from the thymus. PTNs are hardly distinguishable from the thymus in mice probably because they are hidden inside the thymus capsule [16]. Similarly to mice, the human parathymic lymph nodes are small lymphatic organs covered with the thymic capsule but could be isolated only from the cadavers of children up to 2 years of age undergoing post-mortem surgery [17]. Parathymic lymph nodes (PTLNs) in humans have been noted only once in the last century using fetal material [18]. PTNs in humans referred to as internal mammary lymph nodes similarly to mice lay at the lateral borders of the thymus (Fig. 1a). PTNs connected to the thymus by mediastinal connective tissue [19] may have implications with respect to the metastatic spread of tumor cells.

Animals

C3H/HeJ mice (briefly C3H), are used in a wide variety of research areas including cancer research. Male and female C3H mice were kept in a conventional laboratory environment and fed on a semi-synthetic diet (Charles River Mo, Kft, Godollo, Hungary) and tap water ad libitum. Animals received humane care according to the criteria outlined in the UK "Guide for the Care and Use of Laboratory Animals" [20], authorized by the Ethical Committee for Animal Research, University of Debrecen. Each group of mice consisted of two male and two female animals.

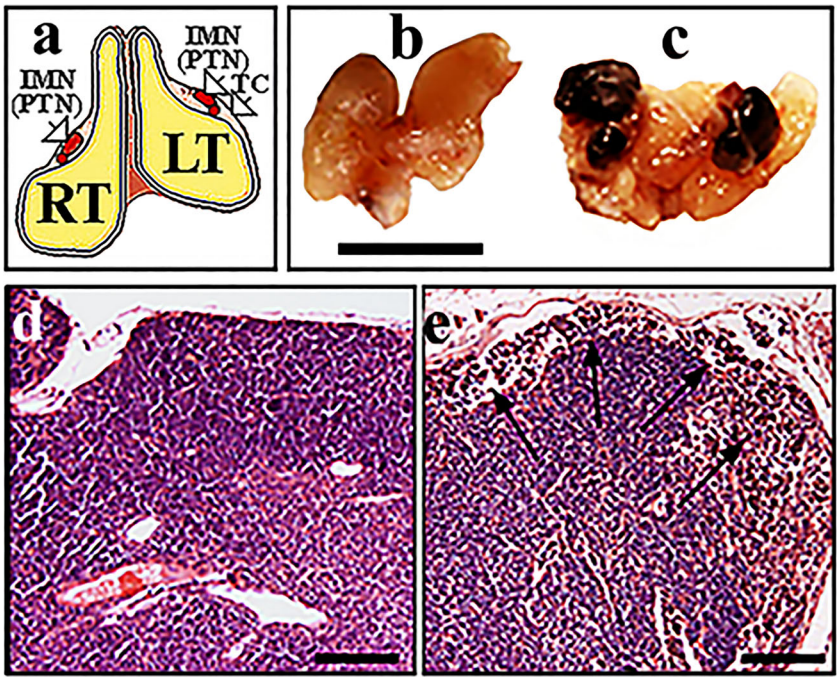

Fig. 1 Mimicking the spread of metastasis in mice by *i.p.* injection of Pelikan Ink. **a** Schematic view of the thymus. RT, right lobe of thymus; LT, left thymic lobe; IMN, internal mammary lymph node; PTN, parahymic lymph node; TC, thymic capsule. **b** Control murine thymus isolated after subjecting mice to saline treatment. **c** PTNs filled with colloid after *i.p.* injection of Pelikan ink. **d** Control hematoxylin-eosin staining of parahymic tissue. **e** Hematoxylin-eosin staining of Pelikan ink containing parathymic lymph nodes. The appearance of colloidal ink particles in parathymic lymph nodes 48 h after *i.p.* administration of Pelikan ink. Black arrows show ink particles in the cortical region of the PTN tissue. Bars: **b**, **c** 0.5 cm each; **d**, **e** 50 μm, each

Tumor Cell Culture, Implantation, and Autopsy

In rat tumor models we have initiated the formation of different types of primary tumors including hepatocarcinoma, nephroblastoma, and myeloid leukemia cell lines followed the path of metastatic spread. and observed an identical metastatic pathway. The idea of implanting SCCVII squamous carcinoma served the purpose to prove that abdominal-thoracal metabolic spread is independent of the implanted cell type and rodent species. Moreover, the similarity of location of PTNs in murine and human thymus could have metabolic and metastatic consequences.

The SCCVII squamous carcinoma cells were chosen because they arose spontaneously in the abdominal wall of a C3H mouse in the laboratory of Dr. H. Suit, Massachusetts General Hospital Boston [21]. Accordingly, C3H mice were infected with these SCC VII cells, propagated in Dulbecco's Modified Eagle's Medium (DMEM-HAM'S F12) (Sigma-Aldrich, Budapest, Hungary). DMEM was supplemented with 2 mM L-glutamine, 23 mM sodium bicarbonate, 100 U/ml penicillin, 100 U/ml streptomycin, 1% non-essential amino acids and 10% heat-inactivated fetal bovine serum. SCC cells (1.5×10^5) suspended in 0.2 ml saline were injected *i.p.* into mice, and after 30 days the mice were sacrificed by cervical dislocation. The abdominal cavity was opened, the primary tumor and the thymus were isolated. When 0.2 ml Pelikan Ink was injected *i.p.* into the peritoneum of the C3H mice to mimick metastatic spread, the experiment lasted for 48 h, before animals were sacrificed and the necropsy performed.

Histology

After necropsy, the thymus including the parathymic lymph nodes and lymph nodes of the mediastinum were fixed in 4% buffered formaldehyde. The specimens were embedded in paraffin, sectioned, stained with hematoxylin-eosin (H&E) and examined under the microscope. Histological studies of the primary tumors grown on the abdominal wall and the tumor-bearing lymph nodes of the thymus were performed at the Department of Pathology, Kenézy University Hospital of Debrecen.

Immunohistochemistry

The immunohistochemical investigation of Cytokeratin 14 (clone: LL002, Biocare Medical, Pacheco, CA, USA) was performed on formalin fixed paraffin embedded mouse tissues to test primary squamous cell carcinoma in thymus tissue, containing squamous cell carcinoma metastasis.

Serial sections of 4 μm thick tissue slices were cut from paraffin blocks. Heat-induced antigen retrieval was performed in EnVision FLEX Target Solution (DAKO, Glostrup, Denmark). Endogenous peroxidase activity was blocked with

3% H_2O_2. As murine monoclonal anti-CK14 antibody we applied the special Vector M.O.M. (mouse-on-mouse) immunodetection kit (Vector Laboratories Inc., Burlingame, CA, USA). Immunoreaction was detected with M.O.M. biotinylated anti-mouse IgG (10 min, room temperature) and then with streptavidin-HRP (Vector Laboratories Inc., Burlingame, CA, USA) (1:300) for 30 min at room temperature. Visualization was performed with 3,3′-diaminobenzidine (DAB), and cell nuclei were counterstained with haematoxylin-eosin. Tissue sections were finally mounted in permanent mounting medium (Histolab, Göteborg, Sweden).

Microscopy

Images from the mouse necropsy were taken with 5-megapixel camera equipped with a LED flash. The imaging of the histological samples was performed with a LEICA DM 2000 microscope.

Results

Delivery of Ink Particles to the Mediastinum and Transmission to Parathymic Lymph Nodes

In the control experiment, saline without ink was administered *i.p.* into 2 male and 2 C3H female mice. The healthy thymus was isolated 48 h after *i.p.* saline injection (Fig. 1b). After 48 h of *i.p.* delivery of Pelikan Ink the parathymic lymph nodes of the murine thymus were packed with the ink. The ink particles accumulated in two larger PTNs at the upper corners of the thymus and in two smaller nodes under the major lymph nodes (Fig. 1c). Earlier the number murine PTNs was not properly determined. The work of Dunn [22] described three to four nodes lying immediately behind the thymus. More importantly, we have found that the four PTNs covered by the thymic capsule were not harbored inside the thymus but extruded and could be isolated by microsurgery. The surgical removal of these lymph nodes will be of medical importance, especially when it comes to the resection of tumorous PTNs before their disruption would cause the spread of metastasis to other, e.g. mammary lymph nodes and could save or extend the life of patients. PTNs may cause errors in the interpretation of experiments where either the thymic tissue or the parathymic lymph nodes alone would be needed [13]. The surgical removal of PTNs could be important in thymus transplantation to avoid transfusion-associated graft versus host disease (GvHD).

From Primary Tumor on Abdominal Wall to Metastasis in Thymus

C3H mice were administered *i.p.* SCC cells (1.5×10^5) and after 30 days the mice were sacrificed and an autopsy was performed. Control thymus is seen in the boxed area of the thorax and after isolation in the inset of Fig. 2a. Few major tumors were observed in the abdominal wall (boxed in Fig. 2b) and many tumor cells as a whitish smear observed in the mediastinum indicated by white arrows. The tumor cells injected into the abdomen caused the enlargement of the thymus (Fig. 2c). The thymus was isolated (inset of 2*c*) and placed into formaldehyde for histological observations.

Histology

Control and metastatic thymus sections were stained with H&E. The overwhelmingly blue staining of the intact thymus with the smaller thymic cells (Fig. 3a) was contrasted by the pinkish color of the tumorous thymus (Fig. 3b). The enlarged large left and the somewhat smaller right thymic lobes are shown in Fig. 2c invaded by the larger tumor cells. The left box in the left lobe was enlarged (Fig. 2b). This panel shows the intrusion of metastasis (right side) into the thymic tissue (left side). The magnified view of the right box of Fig. 3c is seen in Fig. 3d, with a necrotized region inside the metastasis.

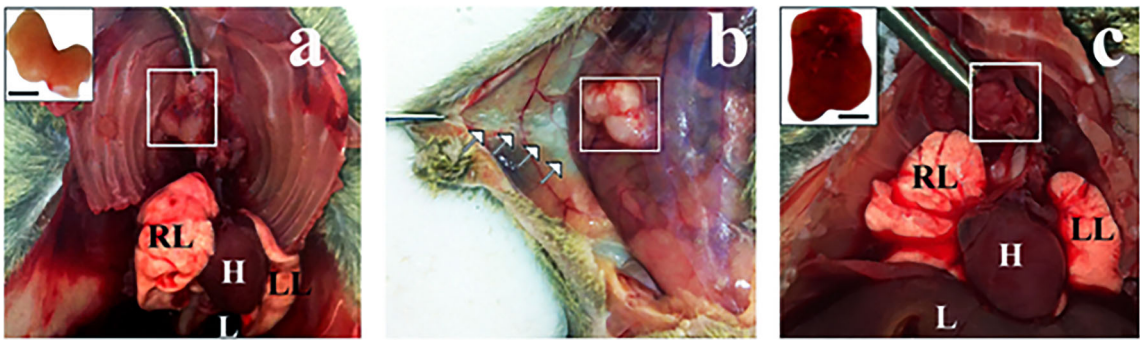

Fig. 2 Primary tumor formation on the abdominal wall and metastasis in the murine thymus. **a** Necropsy of control mouse 30 days after subjection to 0.20 ml *i.p.* saline injection. Box indicates the thymus and inset at the upper left corner the isolated control thymus. Bar, 0,25 cm. RL, right lobe of the lung; LL, left lobe of the lung; H, heart; L, liver. **b** Tumor formation in the thymus 30 days after i.p. SCCVII tumor cell injection. Box: major tumors; white arrows indicate many tumor cells near the site of the injection. **c** Metastatic thymus. Box: enlarged thymus. Inset, isolated tumor-bearing thymus. Scale bar: 0.25 cm

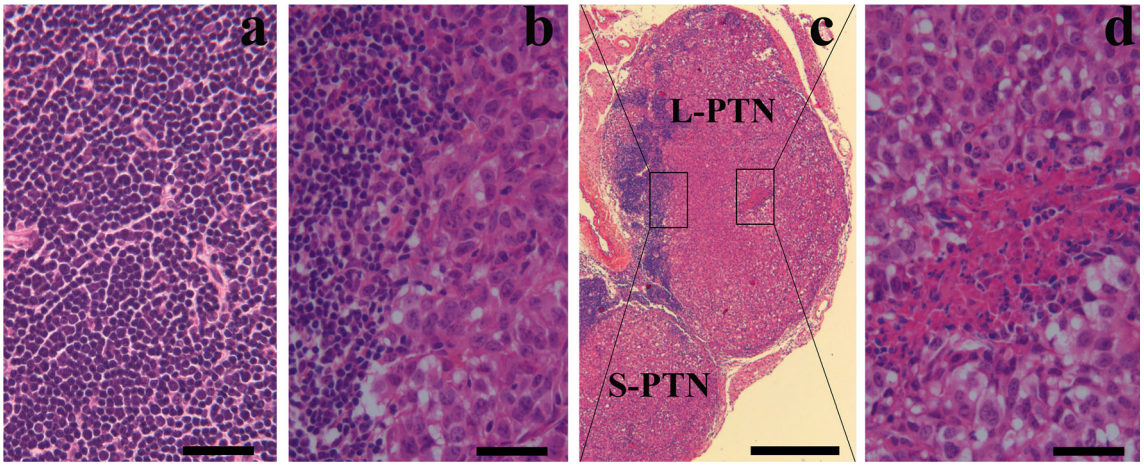

Fig. 3 Hematoxylin-eosin staining of PTN. **a** Magnified view of control PTN (40x). **b** The borderline between the healthy PTN (left) and tumor-bearing tissue (right). **c** Tumor metastasis in the same parathymic lymph node at lower (5x) magnification. Boxes in the middle of the panel were magnified and shown in the left (**b**) and right (**d**) panels. Abbreviations: L-PTN, large parathymic lymph node; S-PTN, small parathymic lymph node. **d** Magnified view of necrosis in the center of the metastatic lymph node (40x). Bars: **a**, **b**, **d**: 50 μm (40x) **c**: 500 μm (5x)

The inner part of the metastasis was necrotized, the outer part disrupted, with blood and tumor cells filtrating into the neighboring tissues. The broken away blood cells along with the released tumor cells are drained to nearby lymph nodes, with parathymic lymph nodes being the sentinel lymph nodes. Parathymic sentinel lymph nodes in the metastatic process behave similarly to primary tumors. Once the immunogenic capacity of the thymus is exhausted, the metastatic tumor growth at the peripheral parts of PTNs is subject to hypoxic angiogenesis and tissue disruptions. The presence of lymph node metastases signifies further metastatic spread and implies poor patient survival.

Immunohistochemical Staining

The immunohistochemical staining was used as a reliable marker to prove the emergence of squamous carcinoma cells inside the PTNs. The control PTN section contained a few thymic microcorpuscles known as Hassall's bodies characteristically present in the medulla of the lobules of the thymus (Fig. 4a). CK14 staining of the tumor-bearing PTN confirmed SCCVII tumor cell positivity in the poorly differentiated tissue (Fig. 4b).

Discussion

Cancer Cell Homing and Metastatic Spread to PTNs in Rats

As far as cancer cell homing is concerned, the liver is the most frequently impacted organ, but less known regarding its metastatic potential to other organs. Liver cancer was and remained the most frequently occurring malignant neoplasm. In a series of 821 malignant neoplasms, 34% metastasized to the liver, 27% to the lungs and much less to other organs [23]. In most of the cases, tumor cells migrated from the colon and rectal cancers through the portal vein supply to the liver. Cancer in the breast, esophagus, stomach, pancreas, kidneys, lung, ovaries, prostate, skin could also spread to the liver.

Our observations regarding tumor growth and migration of tumor cells from abdominal rat tumors are summarized in Fig. 5. In the control experiments no tumor cell implantation was performed (Fig. 5a). Irrespective of the type of tumor cells, implantation generated primary abdominal tumors that grew outward and necrotized inside. After one week of primary tumor growth, HeDe, NeDe, myeloid leukemia (My1De, My2De) [24, 25] cells metastasized and appeared

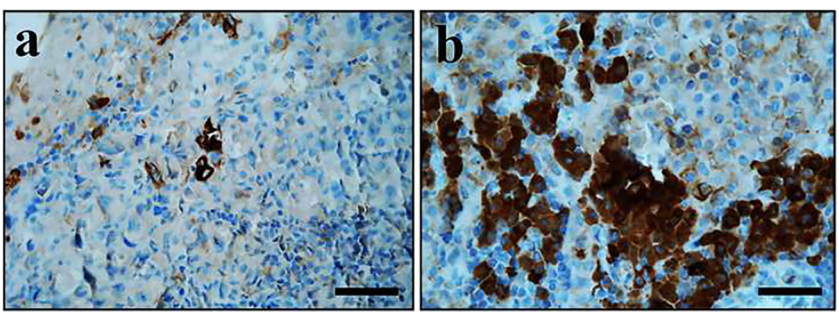

Fig. 4 Immunohistochemical cytokeratin 14 staining of control and SCC VII tumor-bearing PTNs. **a** Control PTN section containing few Hassall's bodies in the middle of the panel. **b** Dark brown stained SCC VII tumor cells in the tissue section of the PTN tumor (40x). Bar, 50 μm each

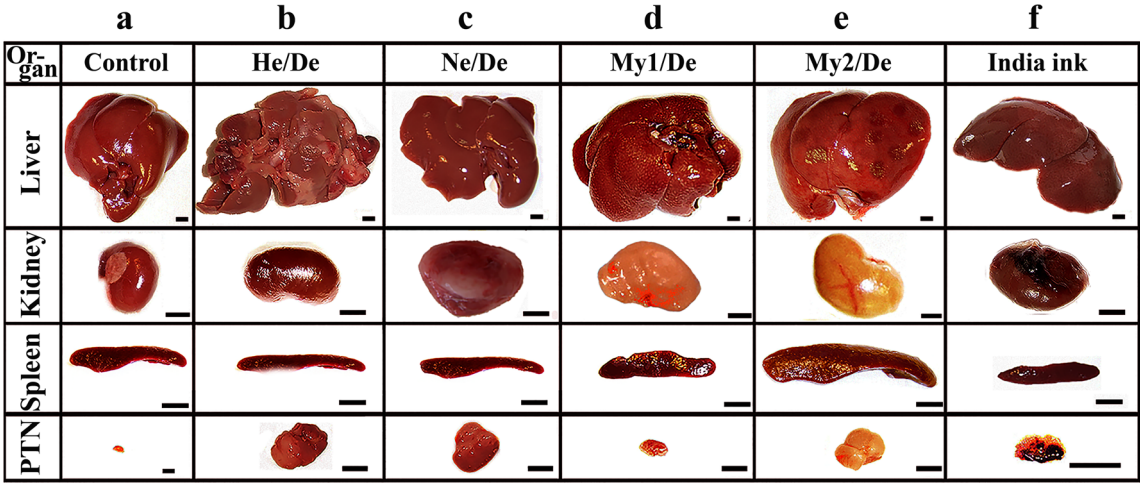

Fig. 5 Abdominal tumor spread to PTNs in rats. Implantation of rat tumor cells inducing abdominal primary tumors (**b**-**e**) or colloidal particles under the renal capsule (**f**). Solid tumors included hepatocarcinoma (HeDe) [24], and nephroblastoma (NeDe) [2] cells or leukemia (My1De, My2De) [25] cells (10^6) placed under the capsule of the left kidney or under the Glisson's capsule of liver in rats. Two weeks after HeDe or NeDe and four weeks after myeloid leukemia cell implantation animals were euthanized. The liver, the impacted left kidney, spleen and parathymic lymph nodes (PTNs) were removed surgically *post-mortem*. **a** Control liver, kidney, spleen and parathymic lymph nodes 14 days after subrenal implantation of saline. **b** Liver hepatocarcinoma formation after HeDe cell (10^6) implantation under the Glisson's capsule. Bottom panel: enlargement of parathymic lymph nodes (PTNs). **c** Nephroblastoma formation in the kidney after subrenal implantation of NeDe cells (10^6). Bottom panel: Enlargement of PTNs. **d** Liver, kidney, spleen, PTN enlargement upon subrenal implantation of My1De cells (10^6). **e** Subrenal implantation of My2De cells (10^6). **f** Implantation under the renal capsule of 0.1 ml 0.1% India ink and appearance 24 h after administration in liver, kidney, spleen and the cortical region of parathymic lymph nodes. Abbreviations: LN, lymph node; PTN, parathymic lymph node. Bar, 0.5 cm each

in the parathymic lymph nodes. After two weeks of implantation, PTNs became enormously enlarged (Fig. 5b-e, bottom panels). The presence of tumor cells in PTNs was confirmed by subrenal reimplantation of tumor-bearing parathymic lymph node slices. Reimplantation induced tumor growth under the renal capsule. Eight days of tumor growth was sufficient to generate metastatic cells in PTNs for the reinduction of primary tumor growth. Hepatocellular carcinoma liver tumor that caused metastasis in parathymic lymph nodes, did not spread to other abdominal organs (kidney, spleen, thymus, lung) (Fig. 5b). Subrenally implanted nephroblastoma cells generated kidney tumor and projected metastasis to PTNs, moderately impacted liver, but did not induce metastasis to other organs (Fig. 5c). Subrenally implanted myeloid leukemia (My1De, My2De) cells beside the primary kidney tumor caused parathymic, liver and spleen metastasis (Fig. 5d and e). Corresponding to the idea that liver is a cancer cell homing center, subrenally implanted India ink particles mimicked metastatic tumor spread, colloidal carbon particles appeared in the liver and deposited in the spleen and in parathymic lymph nodes (Fig. 5f).

The lymphatic tumor spread from the abdominal cavity to the parathymic lymph nodes was mimicked not only by tumor cell implantation, but also by injecting India ink particles directly into the peritoneal cavity. 24 h after implantation colloidal ink particles appeared in PTNs and accumulated in the parathymic lymph nodes within 6 h after *i.p.* injection. Results are in agreement with the observations that in rats the deposition of macrophages laden with colloidal carbon was much less in the lung than in the mediastinal internal mammary and parathymic lymphatics [26]. The passage of carbon-laden particles across the diaphragm to the PTNs indicated that the pleural cavity and lung were not the predominant routes of clearance of the peritoneal lymph.

To avoid implantation and dissemination of tumor cells through the bloodstream, murine squamous carcinoma cells were injected into the peritoneum allowing their development into abdominal tumors, cross through the stomata of the diaphragm and to follow their thoracal route. After 30 days of tumor development mice were sacrificed and autopsy was performed. The spread of tumor cells and its mimicking by the *i.p.* injection of colloidal Pelikan Ink led to to the following conclusions:

1. Tumor cells reached the distantly located parathymic lymph nodes through the thoracal lymph node chain.
2. Murine parathymic lymph nodes consist of two larger and two smaller nodes embedded into but are extruded and can be removed by microsurgery from the thymic tissue.
3. The lymphatic spread of abdominal tumor cells from the effusion is in conformity with earlier observations obtained with hepatocarcinoma, nephroblastoma and myeloid leukemia cells in rat metastatic tumor models. It was shown that fluid or particles injected into the peritoneal cavity may pass directly through the fenestrated basement membrane of mesothelial cells on the peritoneal surface of

the diaphragm, enter the lymphatic pathway between phrenic muscle bundles and empty into the diaphragmatic plexus [27, 28] The diaphragmatic lymphatic plexus drains into the large internal thoracic (mammary) lymphatics, which enter the parathymic lymph nodes.

4. Mediastinal ducts from the parathymic and posterior mediastinal nodes are known to enter the subclavian vein. Metastastatic development returns to and may continue in the bloodstream generating a vicious circle by hematogenic and lymphatic dissemination events reinforcing themselves through the feedback loop.
5. The murine peritoneal-parathymic lymph node route resembles human tumor progression from the abdominal effusion. The growth of abdominal tumors is followed by the release of tumor cells, continues as thoracal metastasis in parathymic lymph nodes and could turn to breast metastasis.
6. The murine model confirmed the validity of results obtained with the rat model suggesting a general mechanism of mammalian metastatic spread from abdominal tumors.

Metastatic Spread from Abdominal Tumor Cells to Thymus in Mice

Tumor cells from abdominal primary tumors can reach distant organs: *i)* by the bloodstream or *ii)* in a lymphatic manner by escaping the primary tumor through the disruptions of the peripheral blood vessels and move from the peritoneal or retroperitoneal cavity across the stomata of the diaphragm to the thoracal cavity and accumulate in the PTNs. In mice, the parathymic nodes are located inside the thymic capsule, whereas in rats, the nodes lie *on* the capsule. Serial sections of lymph nodes showed that they were embedded in the connective tissue of the capsule of the murine thymus [16]. In rat, a thin layer of connective tissue between the thymus and lymph node might have been mistaken for an additional thymic lobule [16].

Lymphatic Drainage of Human Abdominal Tumor Cells to Mammary Lymph Nodes

In conformity with animal experiments, the upper gastrointestinal bleeding and ascites formation of patients is one of the most common complications in the metastatic spread of hepatocellular carcinoma. Variceal ruptures produce copious amount of ascites fluid building up between the two layers of the peritoneum in the abdominal cavity. Beside palliative ascitic drainage known as *paracentesis,* not much has been done to alleviate the discomfort and pain of patients, while the fate of the cancer cells in the peritoneum remained largely unknown. One can expect that the recognition of the close relationship between the thoracic and internal mammary lymph nodes will contribute to early diagnosis and therapy of breast cancer [29].

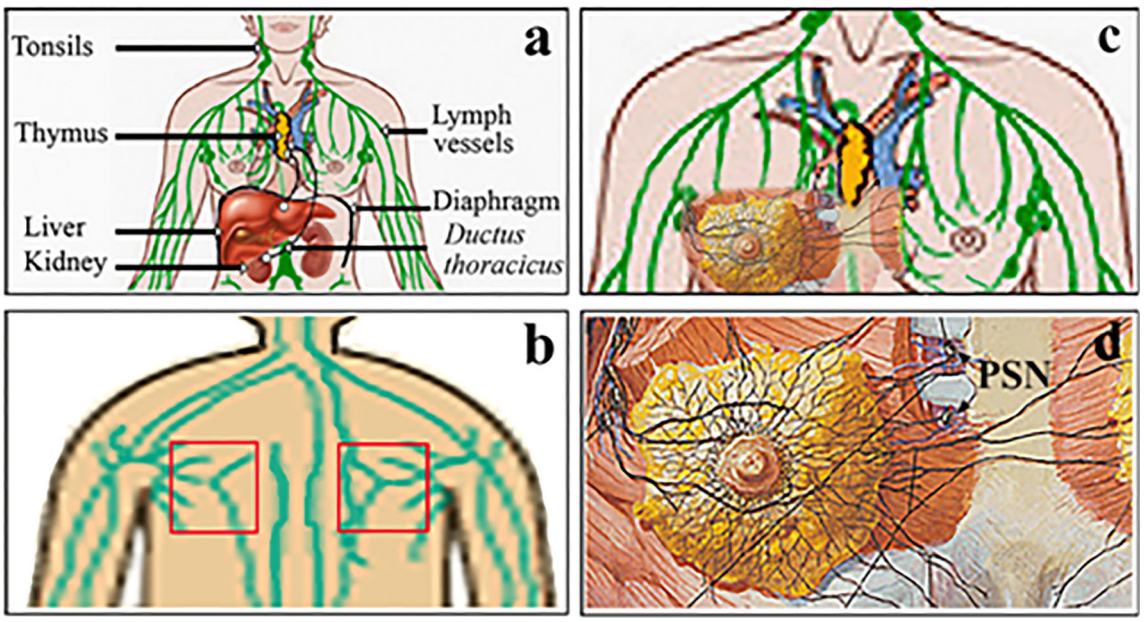

Fig. 6 Metastatic spread of tumor cells from the abdominal to the thoracal cavity. Based on rodent experiments the schemes are extended to human metastatic spread. **a** Tumor cells generating peritoneal (liver) or retroperitoneal (kidney) primary solid tumors, or originating from leukemia cells appear in the thoracal parathymic lymph nodes (curved black arrows). **b** Potential spread of tumor cells through the lymphatic anastomoses from the thoracal lymph node chain to the upstream thoracal, mediastinal and mammary lymph nodes. **c** thoracal to mammary spread of tumor cells, **d** enlarged part in **c** viewing the spread of tumor cells to the breast as a metastatic process. Black arrows in this panel indicate parasternal lymph nodes (PSN)

Several arguments have been listed that supported the idea that cancerous regional lymph nodes among them tumorous mammary lymph nodes are not primary tumors, but the consequence of metastasis [29]. Tumor cells shedded by the primary tumors of the kidney to the retroperitoneum or tumor cells from the liver tumors to the peritoneum cross the diaphragm and appear in the thoracal lymph nodes (Fig. 6a). Tumor cells may spread through the lymphatic anastomoses from the thoracal lymph node chain to the upstream thoracal, mediastinal and mammary lymph nodes (Fig. 6b). Thoracal to mammary spread of tumor cells is anatomically feasible (Fig. 6c). Lymph carrying cancer cells can drain to opposite direction especially when lymphatic blockade occurs that may redirect tumor spread (Fig. 6d).

The idea of spreading tumors cells to the breast is in conformity with the blood supply and lymphatic drainage of the breast:

- Vascular associations:
- blood vessels of the breast exhibit extensive branches and anastomoses,
- anastomoses between medial mammary branches of internal thoracic artery and lateral mammary branches of internal thoracic artery provide direct connection between thoracal and mammary blood supply,
- internal (mammary) thoracic artery located inside thorax laterally to sternum supplies blood to the anterior thorax and medial mammary glands,
- intercostal arteries located in intercostal spaces (between ribs) supply anterior, posterior and lateral thorax and breast.
- Lymphatic associations:
- vascular associations of breast lymph nodes exist between parasternal and thoracic vessels,
- cancer cells tend to spread along lymph passages,
- main lymphatic drainage of the breast (75%) is via axillary nodes, but the remaining drainage is to parasternal nodes,
- unilateral lymphatic blockade my redirect tumor spread,
- lymph carrying cancer cells can drain to opposite direction

Lymphatic vessels of the diaphragm follow the course of their corresponding vessels and terminate not only in the anterior and posterior mediastinal lymphatics, but more importantly in the intercostal and internal mammary glands [30]. This route drains the abdominal tumor cells via the lymphatic vessels from the diaphragm to the mammary glands. These anatomical connections between lymphatic vessels provide the missing link between the abdominal → thoracal → mammary spread of metastasis. In conformity with this idea of metastatic spread of tumor cells it was found that in clinical cases of breast cancer with no axillary involvement and carcinomata of low histological malignancy, there has been a mortality from recurrence of about 25% in the first five years after operation. If the axillary lymphatics were the only path by which carcinoma cells could escape from the breast, this mortality rate would be inexplicable [31]. It was argued that as axilla is not the only path, the internal mammary glands may have been invaded even before carcinoma has reached the axilla. These clinical observations provided experimental evidence to our hypothesis, that breast cancer is not necessarily a primary tumor, but may also be a metastasis [8, 29].

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Authors' Contributions Conception and design: G. Banfalvi, G. Kiraly, G. Nagy, I. Juhasz.

Collection and assembly of data: G. Kiraly, G. Nagy, Z. Hargitai, I. Kovacs.

Data analysis and interpretation: G. Banfalvi, G. Kiraly, G. Nagy, I. Juhasz.

Manuscript writing: G. Banfalvi, G. Kiraly, G. Nagy, I. Juhasz.

Final approval of manuscript: I. Juhasz, G. Banfalvi, G. Nagy.

Accountable for all aspects of the work: G. Banfalvi, I. Juhasz, G. Nagy.

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