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Review Article

Paris Descartes, Anatomy-Scientific Misconduct of Dr. Jean-François UHL:Actin Use Destruction

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ABSTRACT

Research competition in the life science field is intense. Since Dr. Jean-François UHL was too conscious of the disadvantage of delaying the publication of the paper, he fabricated the data and submitted the final draft or revised version of the paper without any confirmation from co-authors including the first author. From this fact, it can not be denied that he neglected authorship of the journal. He published many articles, falsely announcing the result of Actin as D2-40. Leila HAKKAKIAN,technician: Hôpital Necker-Enfants malades, Paris (Necker), faithfully continued to conceal the fact of the use of ACTIN (18/05/2011-) , and finally confessed (22/06/2016). Despite hertestimony, UHL is still silent. In fact there was a course of a process using Actin (Dako, Denmark, M0851 <Réf.>, Dil : 1/300) and D2-40 (Dako, Denmark, M3619 <Réf>, Dil : 1/10). Here Kurobe presents the evidence that Actin was actually used in the experiment.

KEYWORDS

Actin; D2-40

INTRODUCTION

This is an article that "the vascular system of a human normal fetus was observed for the first time with D2-40 ", overturning the established theory [1]. However, this is a research that began with a marker misidentification of Dr. Jean-François UHL. He continued to study in the wrong methodology, concealing the use of Actin, wrote the manuscript incorrectly and posted it to Surgical and Radiologic Anatomy (SRA), ignoring ICMJE (International Committee of Medical Journal Editors.). Shortly before this post, it had been denied acceptance by Anatomical Record (AR) because of violation of ICMJE. Fabrice DUPARC (Editor in Chief: SRA) received a sign of consent to post from Leila (second author) in 2016. It was two years after the publication. And he requested Kurobe a signature (Mail: 21/04/2017, 16:41), but in vain.

TEXT

SMA (Actin)

I. Staining -Overview:

SMA is widely used in the research of blood vessels. If one has no purpose of dyeing new blood vessels and mature blood vessels, and just wants to observe blood vessels, SMA and vWFare tried first. CD 31 and CD 34 are rational as the Physiological functions of the antigen, but when actually dyed, the positive intracellular localization becomes difficult to follow. For example, the positive site should be only on the cell membrane, but in reality, the cytoplasm is also dyed, resulting in a sticky dyeing method, which makes it difficult to judge the physiological state of the cell. It is certainly possible to observe blood vessels with HE staining. However, it depends on the ability of the observer. An experienced pathologist can make an empirical judgment almost instantly. Usually, the collagen fibers are dyed separately by EVG (Elastica van Gieson) staining or the like, and the vessels are extracted and observed. It is impossible to distinguish between blood vessels and lymph vessels. However, it should be noted that immunostaining requires consideration of antigen activation regardless of the kit maker. Moreover, the observation of the vessel can be performed only by a microscope image. The image is an observation image at a low magnification, which is almost similar to macroscopic observation, but it is difficult unless the objective is 20 times or more. Concerning of IHC, a method called DAB color development (BONDMAX Bond Refine DAB Ditection kit; Leica, Australia) is used in this experiment. This technique dyes the nucleus blue with hematoxylin and the antibody positives brown. D2-40 is dyed brown. Since hematoxylin is positively charged, it stains negatively charged nuclei, which is the same as the previous principle. For antibody positivity, the antibody-labeled enzyme degrades the substrate DAB to precipitate a brown pigment. Due to this coloring principle, it seems that the nucleus is blue and the antibody reaction is not dyed other than brown in this experimental system. The antibody reaction can be red or green depending on the type of enzyme labeled on the antibody, but it has not been used in this experimental system. But unfortunately, images are significantly non-specifically overstained overall.

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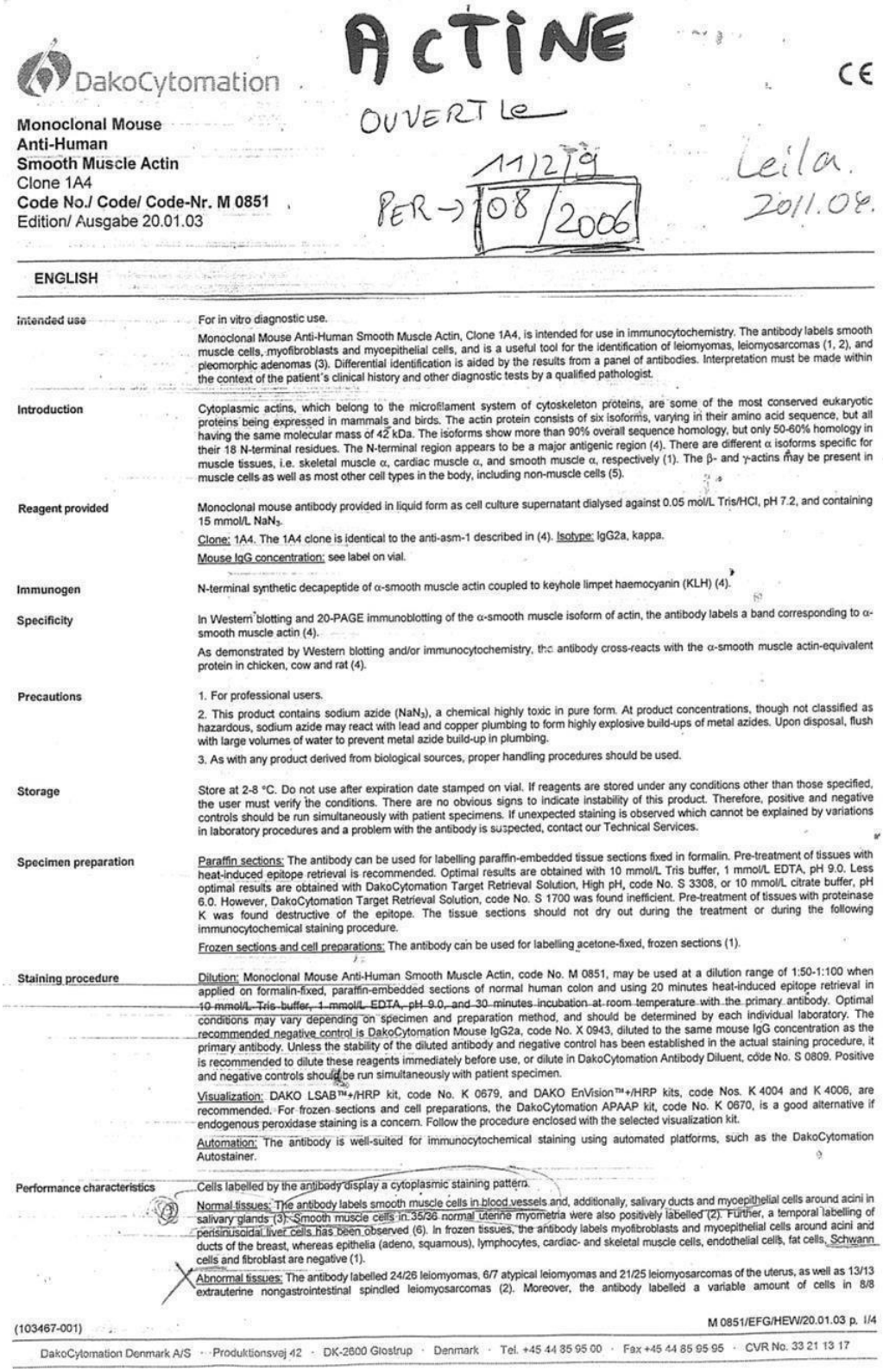
II. Evidence of using Actin in If (Kurobe's Lab Notebook)

A. Actin's protocol handed to Kurobe by Leila HAKKAKIAN, saying; I will use Actin in this experiment. Necker (01/04/2011).



ACTINE

OUVERT le 11/2/9

PER → 08/2006

Leila. 2011.08.

DakoCytomation

CE

Monoclonal Mouse Anti-Human Smooth Muscle Actin
Clone 1A4
Code No./ Code/ Code-Nr. M 0851
Edition/ Ausgabe 20.01.03

ENGLISH

Intended use

For in vitro diagnostic use.

Monoclonal Mouse Anti-Human Smooth Muscle Actin, Clone 1A4, is intended for use in immunocytochemistry. The antibody labels smooth muscle cells, myofibroblasts and myoepithelial cells, and is a useful tool for the identification of leiomyomas, leiomyosarcomas (1, 2), and pleomorphic adenomas (3). Differential identification is aided by the results from a panel of antibodies. Interpretation must be made within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Introduction

Cytoplasmic actins, which belong to the microfilament system of cytoskeleton proteins, are some of the most conserved eukaryotic proteins being expressed in mammals and birds. The actin protein consists of six isoforms, varying in their amino acid sequence, but all having the same molecular mass of 42 kDa. The isoforms show more than 90% overall sequence homology, but only 50-60% homology in their 18 N-terminal residues. The N-terminal region appears to be a major antigenic region (4). There are different α isoforms specific for muscle tissues, i.e. skeletal muscle α, cardiac muscle α, and smooth muscle α, respectively (1). The β- and γ-actins may be present in muscle cells as well as most other cell types in the body, including non-muscle cells (5).

Reagent provided

Monoclonal mouse antibody provided in liquid form as cell culture supernatant dialysed against 0.05 mol/L Tris/HCl, pH 7.2, and containing 15 mmol/L NaN_3.

Clone: 1A4. The 1A4 clone is identical to the anti-asm-1 described in (4). Isotype: IgG2a, kappa.

Mouse IgG concentration: see label on vial.

Immunogen

N-terminal synthetic decapeptide of α-smooth muscle actin coupled to keyhole limpet haemocyanin (KLH) (4).

Specificity

In Western blotting and 20-PAGE immunoblotting of the α-smooth muscle isoform of actin, the antibody labels a band corresponding to α-smooth muscle actin (4).

As demonstrated by Western blotting and/or immunocytochemistry, the antibody cross-reacts with the α-smooth muscle actin-equivalent protein in chicken, cow and rat (4).

Precautions

1. For professional users.
2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.

Storage

Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the user must verify the conditions. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact our Technical Services.

Specimen preparation

Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin. Pre-treatment of tissues with heat-induced epitope retrieval is recommended. Optimal results are obtained with 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. Less optimal results are obtained with DakoCytomation Target Retrieval Solution, High pH, code No. S 3308, or 10 mmol/L citrate buffer, pH 6.0. However, DakoCytomation Target Retrieval Solution, code No. S 1700 was found inefficient. Pre-treatment of tissues with proteinase K was found destructive of the epitope. The tissue sections should not dry out during the treatment or during the following immunocytochemical staining procedure.

Frozen sections and cell preparations: The antibody can be used for labelling acetone-fixed, frozen sections (1).

Staining procedure

Dilution: Monoclonal Mouse Anti-Human Smooth Muscle Actin, code No. M 0851, may be used at a dilution range of 1:50-1:100 when applied on formalin-fixed, paraffin-embedded sections of normal human colon and using 20 minutes heat-induced epitope retrieval in 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0, and 30 minutes incubation at room temperature with the primary antibody. Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. The recommended negative control is DakoCytomation Mouse IgG2a, code No. X 0943, diluted to the same mouse IgG concentration as the primary antibody. Unless the stability of the diluted antibody and negative control has been established in the actual staining procedure, it is recommended to dilute these reagents immediately before use, or dilute in DakoCytomation Antibody Diluent, code No. S 0809. Positive and negative controls should be run simultaneously with patient specimen.

Visualization: DAKO LSAB™+/HRP kit, code No. K 0679, and DAKO EnVision™+/HRP kits, code Nos. K 4004 and K 4006, are recommended. For frozen sections and cell preparations, the DakoCytomation APAAP kit, code No. K 0670, is a good alternative if endogenous peroxidase staining is a concern. Follow the procedure enclosed with the selected visualization kit.

Automation: The antibody is well-suited for immunocytochemical staining using automated platforms, such as the DakoCytomation Autostainer.

Performance characteristics

Cells labelled by the antibody display a cytoplasmic staining pattern.

Normal tissues: The antibody labels smooth muscle cells in blood vessels and, additionally, salivary ducts and myoepithelial cells around acini in salivary glands (3). Smooth muscle cells in 35/36 normal uterine myometria were also positively labelled (2). Further, a temporal labelling of perisinusoidal liver cells has been observed (6). In frozen tissues, the antibody labels myofibroblasts and myoepithelial cells around acini and ducts of the breast, whereas epithelia (adeno, squamous), lymphocytes, cardiac- and skeletal muscle cells, endothelial cells, fat cells, Schwann cells and fibroblast are negative (1).

Abnormal tissues: The antibody labelled 24/26 leiomyomas, 6/7 atypical leiomyomas and 21/25 leiomyosarcomas of the uterus, as well as 13/13 extrauterine nongastrointestinal spindled leiomyosarcomas (2). Moreover, the antibody labelled a variable amount of cells in 8/8

(103467-001) M 0851/EFG/HEW/20.01.03 p. 1/4

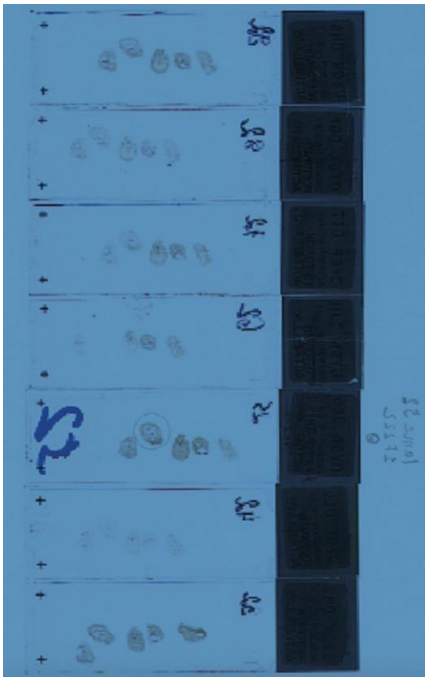
DakoCytomation Denmark A/S · Produktionsvej 42 · DK-2600 Glostrup · Denmark · Tel. +45 44 85 95 00 · Fax +45 44 85 95 95 · CVR No. 33 21 13 17

B. 7 Actin slides which Kurobe received from Leila HAKKAKIAN, as the result of the Actin experiment: Necker (06/05/2011).

*Slide number 52: It is not 25 but 52. This is the error of Leila HAKKAKIAN

C. 7 Actin Slides by Leila, which Kurobe showed Prof. Yves FRAPART in his office. He did confirm them. Univ. (18/10/2013)

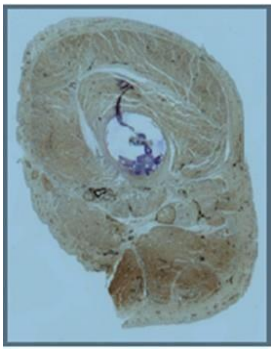
C-1



C-2

C-3

C-4 1F222



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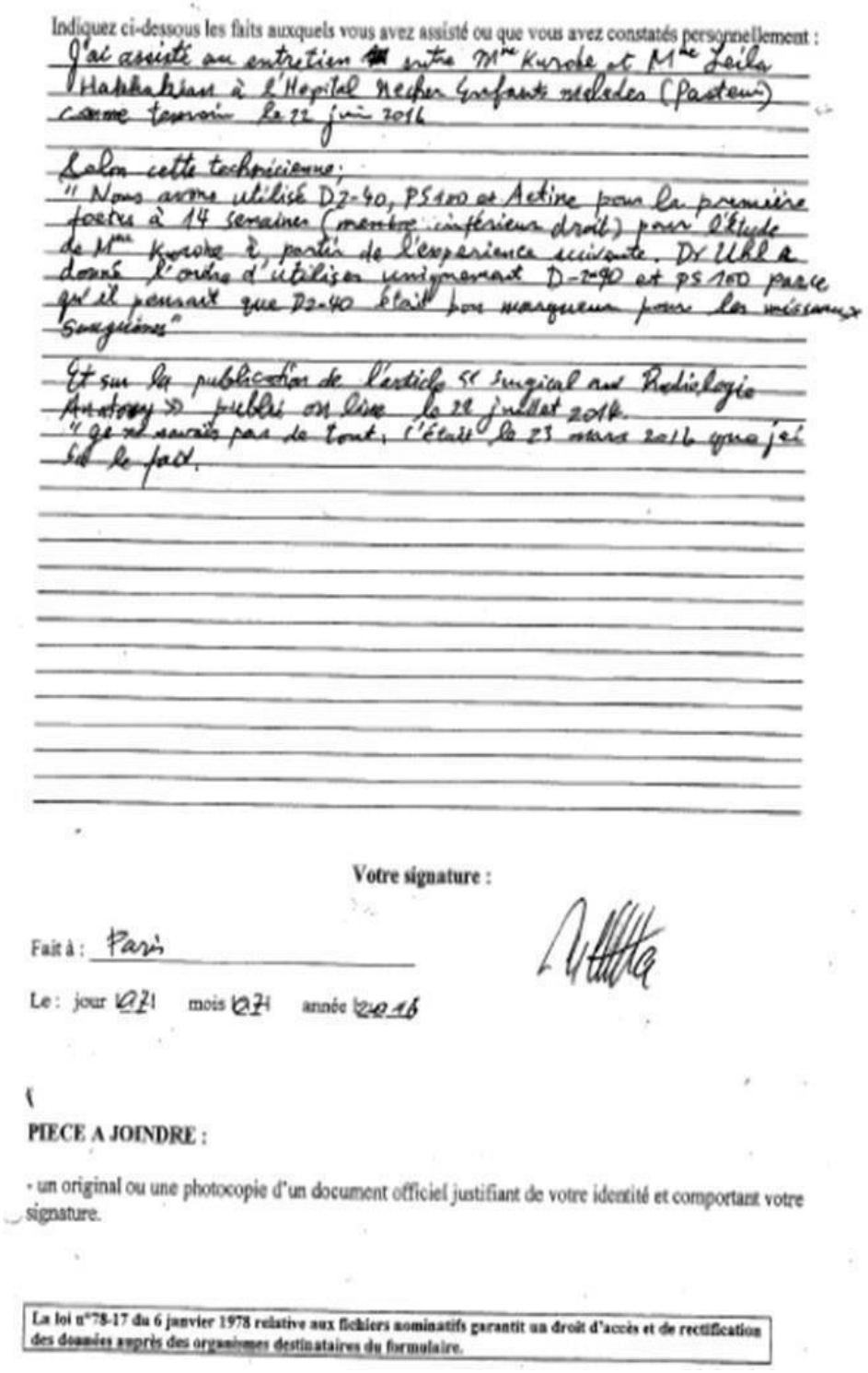
D. Testimony by URATA Ryoichi (Official witness): Tribunal Certificate: MINISTERE DE LA JUSTICE ; ATTESTATION, Necker (07/07/2016).



Indiquez ci-dessous les faits auxquels vous avez assisté ou que vous avez constatés personnellement :
J'ai assisté au entretien entre Mme Kurobe et Mlle Leila Hakkakian à l'Hopital Necker Enfants malades (Pasteur) comme temoin le 22 juin 2016

Selon cette technicienne,
" Nous avons utilisé D2-40, PS100 et Actine pour la première fois à 14 semaines (membre inférieur droit) pour l'étude de Mme Kurobe à partir de l'experience suivante. Dr UHL a donné l'ordre d'utiliser uniquement D-2-40 et PS100 parce qu'il pensait que D2-40 était bon marqueur pour les vaisseaux sanguins"

Et sur la publication de l'article " Surgical and Radiologic Anatomy " publié on line le 22 juillet 2016.
" Je ne savais pas de tout, l'Etat le 23 mars 2016 que j'ai fait le fact.

Votre signature :

Fait à : Paris

Le : jour 07 mois 07 année 2016

PIECE A JOINDRE :

- un original ou une photocopie d'un document officiel justifiant de votre identité et comportant votre signature.

La loi n°78-17 du 6 janvier 1978 relative aux fichiers nominatifs garantit un droit d'accès et de rectification des données auprès des organismes destinataires du formulaire.

He attended Kurobe's interview with Leila HAKKAKIAN, at the Hospital Necker-Enfants malades as a witness on June 22, 2016. And he wrote down the words of Leila HAKKAKIAN

*ON IHC

"We used D2-40, PS100 and Actin for the first fetus at 14 weeks (right lower limb) for Kurobe's study. From the following experiment, Dr. UHL gave order to use only D2-40 and PS100 because he had thought D2-40 to be a good marker for blood vessels."

*On SRA

"I didn't know the publication of the article. It was March 23, 2016 that I knew the fact."

E: Confirmation of 7 Actin slides by Nathalie (IHC-labo), Moslemi AMINE (Interne en Médecine) and Armelle (Secrétary: Bureau d'Anatomie Pathologique, Foeto Pathologie) : Necker. (03/09/2020)

E-1

E-3

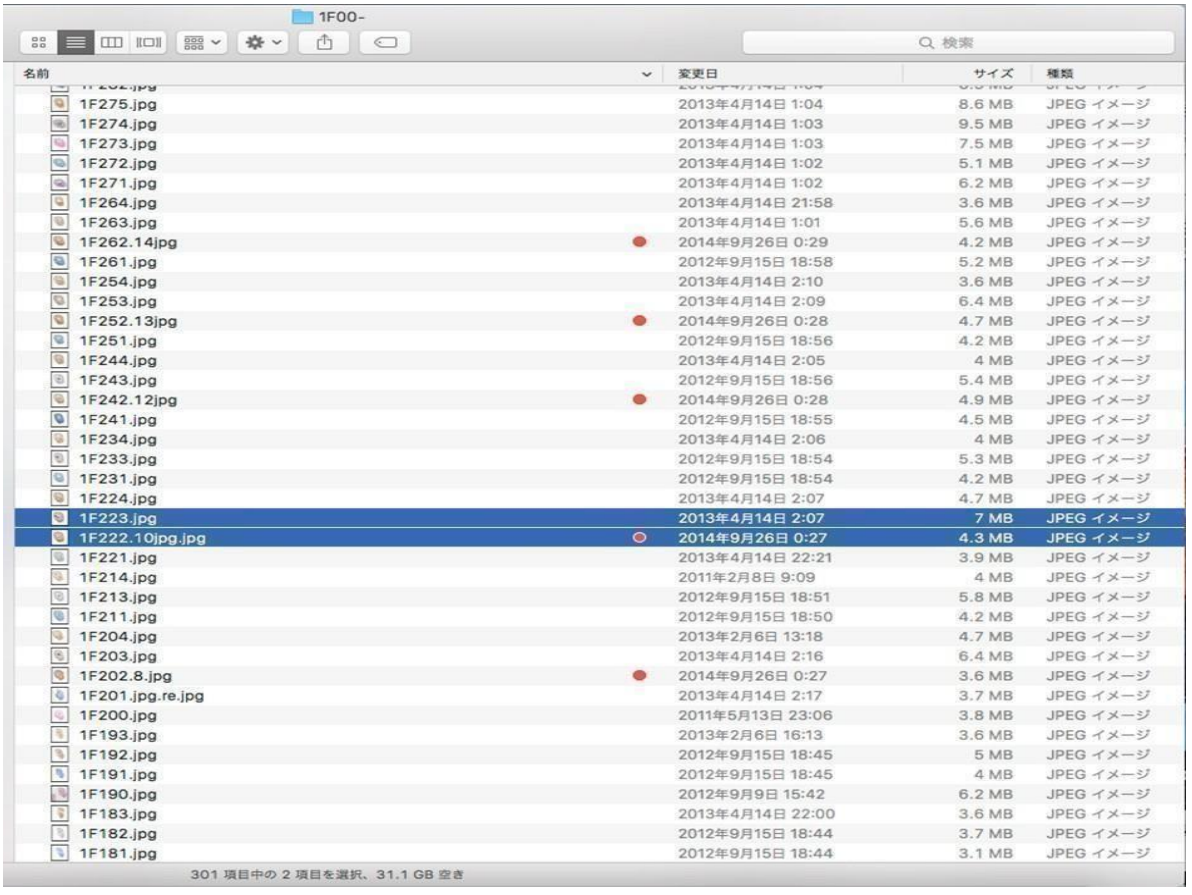
E-4

E-2

F. List of 1F, (Original:15/05/2011), For image-clicking to confirm, some have a new date.

F-1. (a)

1F222 (Actin), 1F223 (D2-40)



F-2.(b)
7 Actin Slides (yellow): Slide No. 52, 1F222

1F Lame No.	Coloration. Immuno Marquage	SERIE 1	SERIE 2	SERIE 3	SERIE 4	SERIE 5
00	HES	1F00	1F170	1F340	1F510	1F680
01	TRIC	1F01	1F171	1F341	1F511	1F681
02	D2-40	1F02	1F172	1F342	1F512	1F682
03	PS100	1F03	1F173	1F343	1F513	1F683
10	HES	1F10	1F180	1F350	1F520	1F690
11	TRIC	1F11	1F181	1F351	1F521	1F691
12	D2-40	1F12	1F182	1F352	1F522	1F692
13	PS100	1F13	1F183	1F353	1F523	1F693
20	HES	1F20	1F190	1F360	1F530	1F700
21	TRIC	1F21	1F191	1F361	1F531	1F701
22	D2-40	1F22	1F192	1F362	1F532	1F702
23	PS100	1F23	1F193	1F363	1F533	1F703
30	HES	1F30	1F200	1F370	1F540	1F710
31	TRIC	1F31	1F201	1F371	1F541	1F711
32	ACTIN	1F32	1F202	1F372	1F542	1F712
33	D2-40	1F33	1F203	1F373	1F543	1F713
34	PS100	1F34	1F204	1F374	1F544	1F714
41	TRIC	1F41	1F211	1F381	1F551	1F721
42	ACTIN	1F42	1F212	1F382	1F552	1F722
43	D2-40	1F43	1F213	1F383	1F553	1F723
44	PS100	1F44	1F214	1F1384	1F554	1F724
51	TRIC	1F51	1F221	1F391	1F561	1F731
52	ACTIN	1F52	1F222	1F392	1F562	1F732
53	D2-40	1F53	1F223	1F393	1F563	1F733
54	PS100	1F54	1F224	1F394	1F564	1F734
61	TRIC	1F61	1F231	1F401	1F571	1F741
62	ACTIN	1F62	1F232	1F402	1F572	1F742
63	D2-40	1F63	1F233	1F403	1F573	1F743
64	PS100	1F64	1F234	1F404	1F574	1F744
71	TRIC	1F71	1F241	1F411	1F581	1F751
72	ACTIN	1F72	1F242	1F412	1F582	1F752
73	D2-40	1F73	1F243	1F413	1F583	1F753
74	PS100	1F74	1F244	1F414	1F584	1F754
81	TRIC	1F81	1F251	1F421	1F591	1F761
82	ACTIN	1F82	1F252	1F422	1F592	11762
83	D2-40	1F83	1F253	1F423	1F593	1F763
84	PS100	1F84	1F254	1F424	1F594	1F764
91	TRIC	1F91	1F261	1F431	1F601	1F771
92	ACTIN	1F92	1F262	1F432	1F602	1F772
93	D2-40	1F93	1F263	1F433	1F603	1F773
94	PS100	1F94	1F264	1F434	1F604	1F774

CONCLUSION

D2-40 (Podoplanin), a lymphatic and mesothelial marker, could not be expressed in normal blood vesssels. Therefore, there is a strong concern for the main purport, restoring of the venous system. It specially reacts with the endothelial cells of lymph channels, not with noncancerous normal vessels. Since the lymph channels become differentiated from veins and run alongside the veins while maintaining a distance which is not too close but not too remote, there are extremely height risk that it might be considered that lymph channels are veins.

Note

This time, Susana M. Chuva de Sousa Lopes offered to do an IHC confirmation experiments for KUROBE and she showed the differences between D2-40 (PDPN), KIT and SMA. <Kidney Vascularization Staining : 15.2 weeks of gestation, etc.> 11-01-2018 and 01-02-2018. Then she used different combinations of PECAM1, PDPN (D2-40), ACTA2 (aSMA) and SOX17. As for 1F222, she said first, <In the artery, the staining is very thick and looks very much indeed like typical aSMA staining. There may be some confusion regarding the use of aSMA and D2-40 in that particular slide.> She was correct because it was the error of Dr. UHL. The use of specific markers is the basis of immunohistochimie.

ACKNOWLEDGEMENT

I would like to thank Kohei SHIOTA: ex vice President of Kyoto University, ex Director of Congenital Anomaly Research Center, Kyoto University Graduate School of Medicine, Kyoto, Japan, and Taichi EZAKI : ex Professor and Chairman of Department of Anatomy and Developmental Biology, School of Medicine, Tokyo Women's Medical University, Tokyo, Japan, Chairman of the Japanese Society of Lymphology, Nagano, Japan. And to Susana M. Chuva de Sousa Lopes, Associate Prof. Dept. of Anatomy and Embryology , Leiden University Medical Center, Leiden, Neitherlands.

REFERENCES

1. Kurobe. N, Hakkakian.L, Chahim.M, Delmas V, Vekemans M, UHL JF: Three -Dimensional reconstruction of the lower limb's venous system in human fetuses using the copmuter-assisted anatomical dissection (CAAD) technique. 2015, 37 (3) : 231- 238, Surg Radiol Anat DOI 10 1007/s00276-014-1350 2

2. UHL J.F : Focus on venous embryogenesis of the human lower limbs. : CAAD ; A New Technique to build 3D Models depicting the Main Steps of Venous Embryogenesis. Phlebolymphology.2015, 22 (2), <55-63, Figure 9, immuno markers for nerves and vessels in a 13-week. P.59 >

3. UhL.J.F, Gillot. C: A New tool to study the 3D Venous Anatomy of the Human Embryo : The Computer-Assisted Anatomical Dissection. J Vasc Surg Venous Lymphat Disord, 2014, 2(1) :111-112 Doi :10.1016/j.jvsv.2013.10.025

4. Uhl.J.F, Chahim.M, Cros. F, Ouchene. A :3D modeling of the vascular system, Vascular Research, Vascular Branching, JTAVR, 2016, 1(1):51-58 presented to: the Hemodyn 2015, Nov 2015, Naples, Italy submitted: Apr 30, 2016, accepted: Jun 19, 2016, EPub Ahead of Print: Nov 30, 2016, published: Dec 31, 2016

5. Uhl.J. F, Prat.G, Costi.D, Ovelar. J. A, Scarpelli. F, Ruiz. C, Lorea B: Modelado 3D del sistema vascular, Flebologia. 2018, 44(1) ; 17-27 Revista Flebologia, Organo Oficial de la Sociedad Argentina de Flebologia y Linfologia-Ano 44/No 1/Junio de 2018 :44/17-27

6. KUROBE. N, (2020) The use of D2-40 for Blood Vessels Modeling is inappropriate, JBL-20-6528, jan. 27, 2020

7. KUROBE. N, (2020) Use of D2-40 as an IHC marker is inappropriate for 3D Reconstruction of the Venous System. AnatPhysiol 10:322.

8. KUROBE. N, (2020) Embryological Evidence disproving Detectability of Blood Vessels using D2-40. Anat Physiol 10:324. doi: 10.35248/2161-0940.20.10.324