Report on the analysis of subcutaneous nodules induced by the injection of iPS cells

METHODOLOGY

Each male Nod SCID gamma mouse was injected subcutaneously, contralaterally, with two different cell lines, including a control line.

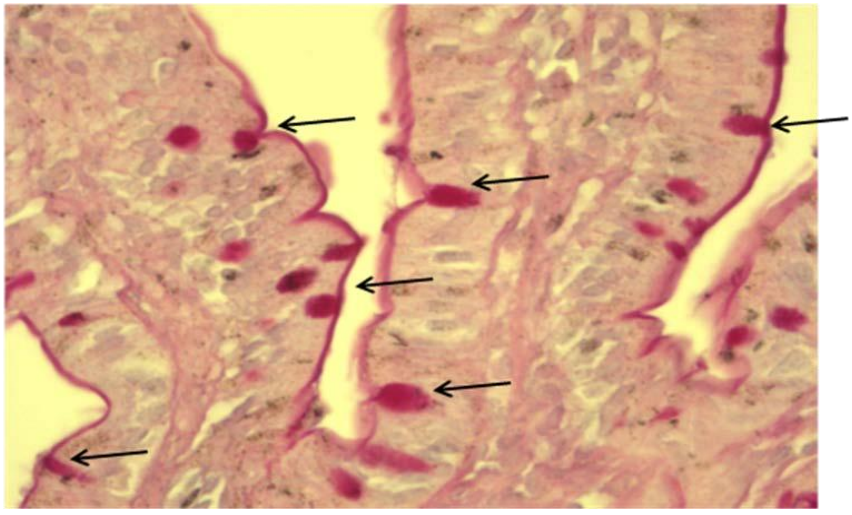
3,106 cells were grafted and tumor samples were taken 41 days post-graft.

The injected cells were either in clusters or isolated cells to test for the best take.

The nodules that developed following subcutaneous injection of the iPS cells were removed, fixed in formalin, cut, embedded in paraffin, and analyzed after staining.

Microscopic analysis of two or three sections of the nodule was performed to identify histological structures derived from the three embryonic layers, in order to confirm the pluripotency of the iPS cells.

From each block, two slides were stained, one with Hematoxylin-Eosin-Saffron (HES), a classic topographic stain, and the other with PAS (Periodic Acid Schiff), a special stain designed to highlight the mucus of endodermal-derived goblet cells. An additional slide (small intestine slide 1203-0084) was included in the PAS-stained batch as a control and to highlight the goblet cells (see following figure).



Coloration PAS
Intestin grêle
Mucus (flèche noire) des cellules mucipares caliciformes
Fort grossissement (g=40)

Group 1: LEB_M

Injection into two NOD-SCID gamma mice, mice A and B. Location: subcutaneous region on the right.
Injected line: iPS Cluster Sendai late passage Number of cells injected: 3 million cells

MACROSCOPY

Observation of dense tissue nodules, measuring mouse A = 451 mm3 => 12 x 8.5 mm2 (LxW) Mouse B = 505 mm3 => 12 x 9 mm2

MICROSCOPY

The sample consists of one or more well-defined nodules developed in the adipose tissue of the hypodermis. These nodules are variably dense with cells, sometimes with cystic areas but without tissue necrosis. Numerous embryonic structures are observed, separated by undifferentiated embryonic mesenchymal cells. The cells show signs of malignancy, with numerous mitoses and cell clusters with loss of polarity.
The majority of cells are arranged in neuroectodermal structures (neural tube primordia, sometimes accompanied by clearly pigmented melanocytes), associated with ectodermal structures (dental primordia +/- sebaceous glands).
Mesodermal structures are also observed, present in smaller quantities, such as smooth muscle, cartilage, and, more rarely, bone.
Endodermal structures were sought. They are very rare. Their observation required the use of PAS staining to visualize mucus, observed as a small fuchsia-pink lake rather apically in the cell.

Conclusion
The pluripotent iPS cells injected subcutaneously induced the formation of a subcutaneous teratocarcinoma.
All three germ layers are present, with ectoderm derivatives predominating, accompanied by smaller quantities of mesoderm derivatives. Endoderm-derived cells are observed only very rarely.

groupe 1 : LEB_M

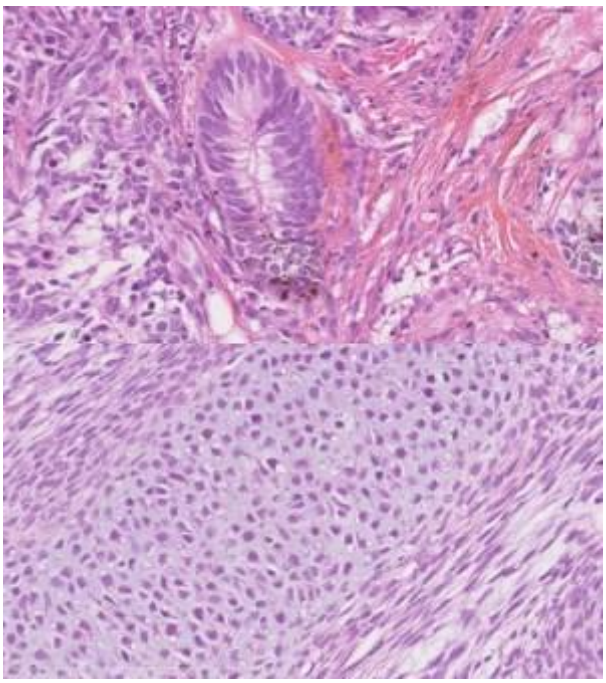
	ECTODERME					
	ébauche dentaire	Glandes sébacées	Formation type tube neural	Neurones : Mélanocytes	Cellules de crête neurale	
LEB_M	×	×	×	×	×	

	MESODERME					
	muscle muscle lisse	strié	Tissu adipeux	cartilage	Os	structures testis comme
LEB_M	×			×	×	

	ENDODERME					
	épithélium respiratoire	épithélium digestif				
LEB_M		×				

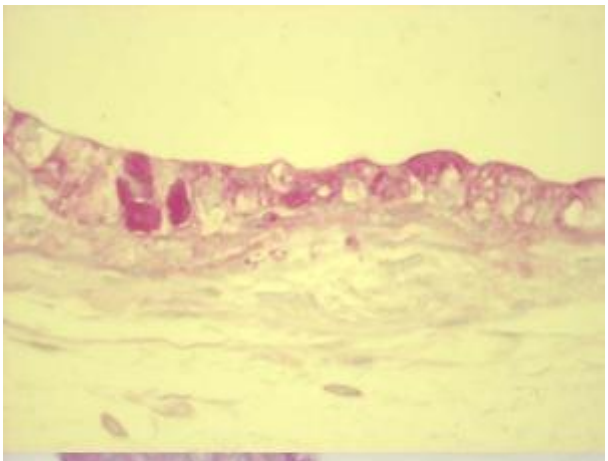
	BILAN					
	Ectoderme	mésoderme	endoderme			
LEB_M	×	×	×			

Figure groupe 1 : LEB_M

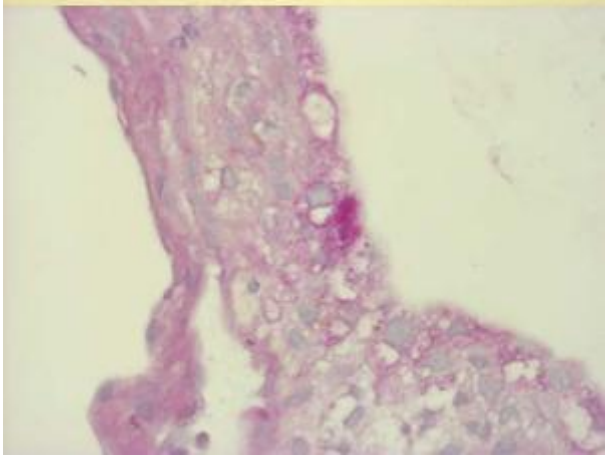


Dérivés neuro-ectodermiques : tube neural et mélanocytes
boiteux g = 40

Dérivé mésodermique cartilage
lame g=40



Dérivé endodermique épithélium intestinal
coloration de PAS
Boiteux g=40



g=40 coloration de PAS

groupe 2 : PIN_L

Injection into a NOD-SCID gamma mouse, and location: subcutaneous region on the right. Injected cell line: iPS SCED Sendai Late passage Number of cells injected: 3 million cells

MACROSCOPIE

Observation of dense tissue nodules, measuring for the mice = 293 mm3 => 10 x 7,5 mm2 (Lxl)

MICROSCOPY

The sample consists of one or more well-defined nodules developed in the adipose tissue of the hypodermis. These nodules are variably dense with cells, sometimes with cystic areas but without tissue necrosis. Numerous embryonic structures are observed, separated by undifferentiated embryonic mesenchymal cells. The cells show signs of malignancy, with numerous mitoses and cell clusters with loss of polarity.

Most of the cells are arranged in neuroectodermal structures (neural tube primordia, sometimes accompanied by clearly pigmented melanocytes), associated with ectodermal structures (dental primordia).

Mesodermal structures are also observed, present in smaller quantities, such as smooth muscle, cartilage, and, more rarely, bone.

Endodermal structures were sought. They are very rare. Their observation required the use of PAS staining to visualize mucus, observed as a small fuchsia-pink lake rather apically in the cell.

Conclusion

Pluripotent iPS cells injected subcutaneously induced the formation of a subcutaneous teratocarcinoma.All three germ layers are present, with ectoderm derivatives predominating, accompanied by smaller quantities of mesoderm derivatives. Endoderm-derived cells are observed only very rarely.

groupe 2 : PIN_L

Analyse histologique de tératomes induits par injection d'iPSC - pour F. Becker & J-M Lemaître- F. Bernex le 03-03-2015

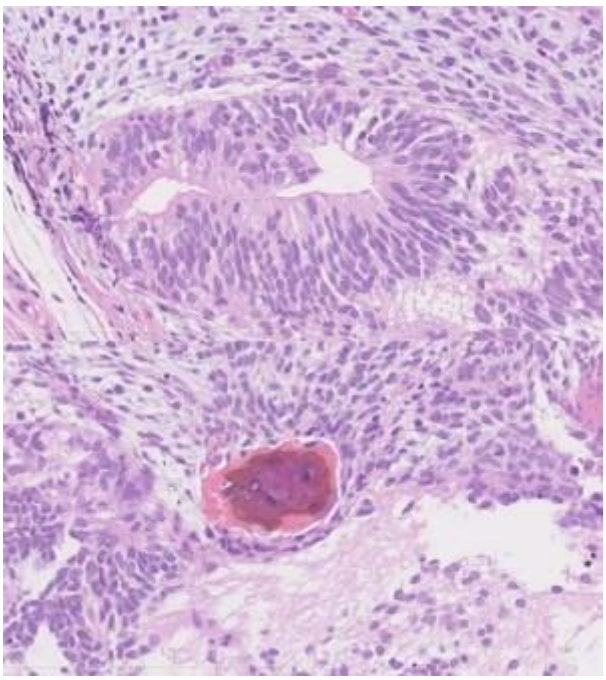
	ECTODERME					
	ébauche dentaire	Glandes sébacées	Formation type tube neural	Neurones : Mélanocytes	Cellules de crête neurale	
PIN_L	x	x	x	x	x	

	MESODERME					
	muscle muscle lisse	strié	Tissu adipeux	cartilage	Os	structures testis comme
PIN_L	x			x	x	

	ENDODERME					
	épithélium respiratoire	épithélium digestif				
PIN_L		x				

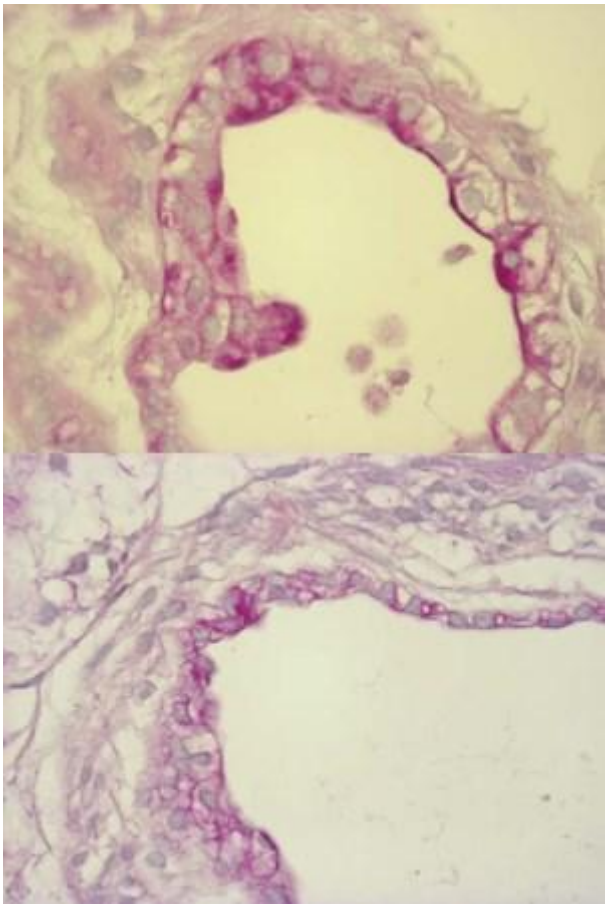
	BILAN					
	Ectoderme	mésoderme	endoderme			
PIN_L	x	x	x			

Figure groupe 2 : PIN_L



Derivé neuro-ectodermique : tube neural
lame 1507-008 g=40

Dérivé mésodermique os
lame 1507-008 g=40



Dérivé endodermique épithélium intestinal
boiteux 1507-008, g=40

lame 1507-0010, g=40 PAS

groupe 3 : COL-T

Injection into a NOD-SCID gamma mouse, mouse C. Location: subcutaneous region on the right.

Injected line: iPS Cluster Sendai Early passage

MACROSCOPY

Observation of dense tissue nodules, measuring for mouse C = 702 mm3 => 13.5 x 10 mm2 (LxW)

MICROSCOPY

The sample consists of one or more well-defined nodules developed in the adipose tissue of the hypodermis. These nodules are variably dense with cells, sometimes with frequent cystic areas and rare areas of tissue necrosis. Numerous embryonic structures are observed, separated by undifferentiated embryonic mesenchymal cells. The cells show signs of malignancy, with numerous mitoses and cell clusters with loss of polarity.

The majority of cells are arranged in neuroectodermal structures (neural tube primordia, sometimes accompanied by clearly pigmented melanocytes, a few neuronal clusters), associated with ectodermal structures (dental primordia).

Mesodermal structures are also observed, present in smaller quantities, such as smooth muscle, cartilage, and more rarely bone.

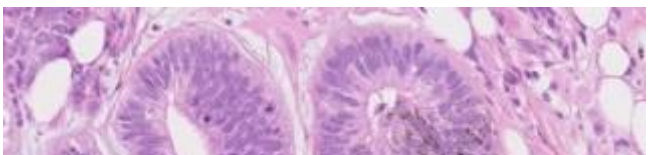
Endodermal structures were sought. They are very rare. Their observation required the use of PAS staining to visualize the mucus, observed as a small fuchsia-pink lake rather apically in the cell.

Conclusion

The pluripotent iPS cells injected subcutaneously induced the formation of a subcutaneous teratocarcinoma.

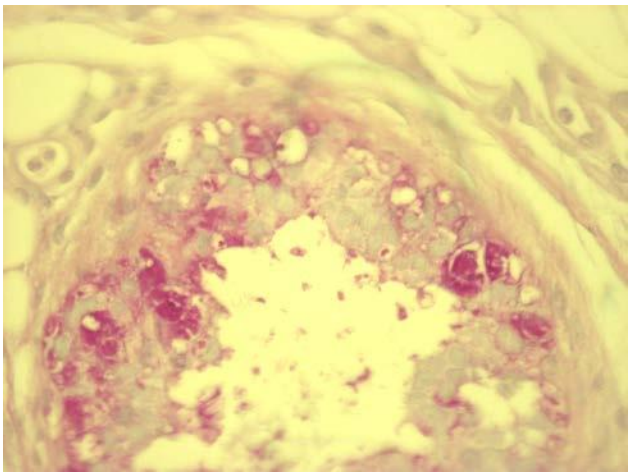
All three germ layers are present, with ectoderm derivatives predominating, accompanied by smaller quantities of mesoderm derivatives. Endoderm-derived cells are observed only very rarely.

Figure groupe 3 : COL-T



Dérivés neuro-ectodermiques : tube neural et mélanocytes
g = 40

Dérivé mésodermique cartilage,
g=20



Dérivé endodermique Épithélium intestinal
g=40

coloration de PAS

Group 4: MAR_N

Injection into three NOD-SCID gamma mice: mice A, B, and C. Location: subcutaneous region on the left. Injected line: iPS MAR_N Number of cells injected: 3 million cells

MACROSCOPY

Observation of dense tissue nodules, measuring for mouse A = 484 mm3 => 11.5 x 9 mm2 (LxW)

MICROSCOPY

The sample consists of one or more well-defined nodules developed in the adipose tissue of the hypodermis. These nodules are variably dense with cells, sometimes with cystic areas, and rare areas of tissue necrosis. Numerous embryonic structures are observed, separated by undifferentiated embryonic mesenchymal cells. The cells show signs of malignancy, with numerous mitoses and cell clusters with loss of polarity.

Most of the cells are arranged in neuroectodermal structures (neural tube primordia, sometimes accompanied by clearly pigmented melanocytes), associated with ectodermal structures (tooth primordia +/- sebaceous glands).

Mesodermal structures are also observed, present in smaller quantities, such as smooth muscle and, more rarely, bone.

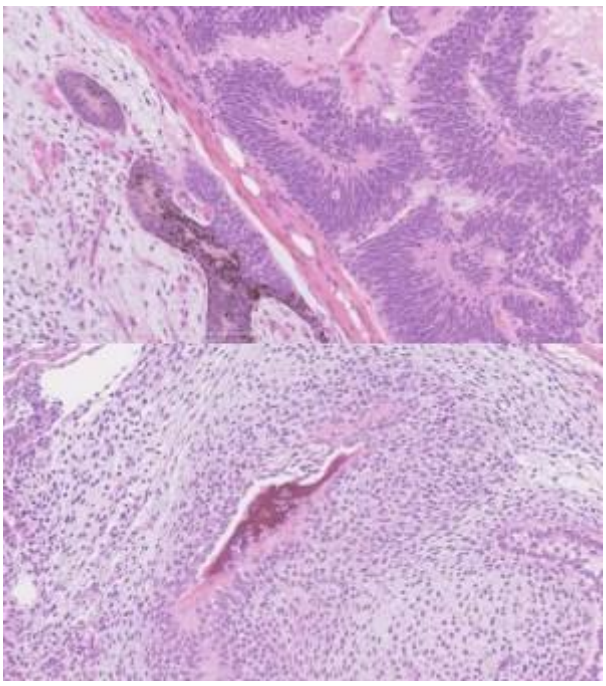
Endodermal structures were sought. They are very rare. Their observation required the use of PAS staining to visualize the mucus, observed as a small fuchsia-pink lake rather in the apical position in the cell.

Conclusion

The pluripotent iPS cells injected subcutaneously induced the formation of a subcutaneous teratocarcinoma.

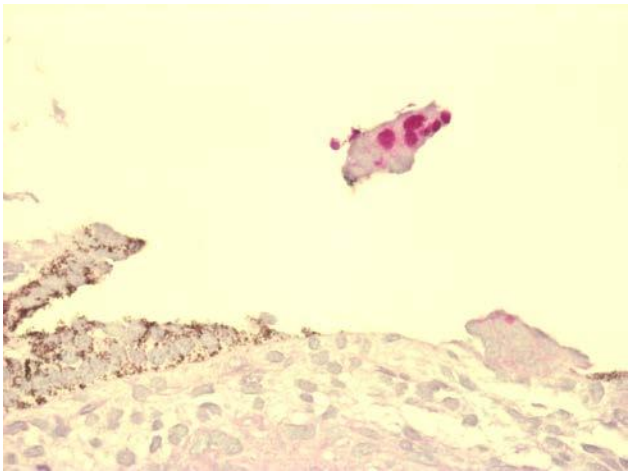
All three germ layers are present, with ectoderm derivatives predominating, accompanied by smaller quantities of mesoderm derivatives. Endoderm-derived cells are observed only very rarely.

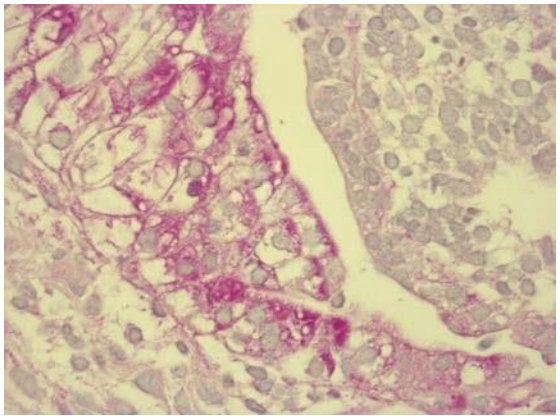
Figure groupe 4 : MAR_N

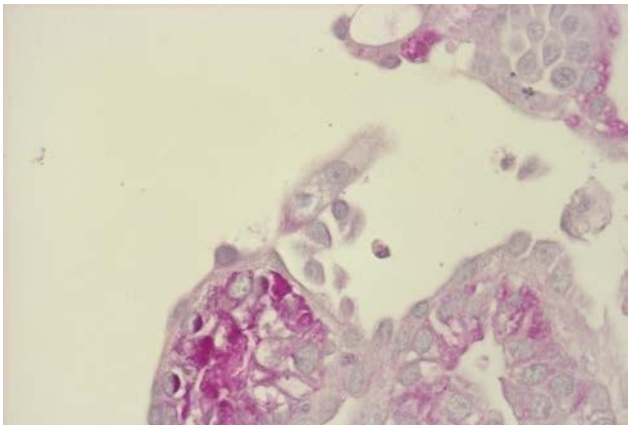


Dérivés neuro-ectodermiques : tube neural et mélanocytes
g = 20

Dérivé mésodermique : os
lame 1507-0005, g=20







Dérivé endodermique épithélium intestinal
Coloration de PAS, lame 1507-005, g=40

lame 1507-007, g=40 coloration de PAS

Lame 1507-003, g=40 coloration de PAS

groupe 5 : VER_C

Injection into a NOD-SCID gamma mouse. Location: subcutaneous region on the left. Injected line: VER-C. Late passage Number of cells injected: 3 million cells

MACROSCOPIE

Observation of dense tissue nodules, measuring for the mouse D= 1,273 mm^3 => 17 x 12 mm^2 (LxW)

MICROSCOPIE

The sample consists of one or more well-defined nodules developed in the adipose tissue of the hypodermis. These nodules are variably dense with cells, sometimes with cystic areas and rare areas of tissue necrosis. Numerous embryonic structures are observed, separated by undifferentiated embryonic mesenchymal cells. The cells show signs of malignancy, with numerous mitoses and cell clusters with loss of polarity.

Most of the cells are arranged in neuroectodermal structures (neural tube primordia, sometimes accompanied by clearly pigmented melanocytes), associated with ectodermal structures (tooth primordia +/- sebaceous glands).

Mesodermal structures are also observed, present in smaller quantities, such as smooth muscle and, more rarely, cartilage.

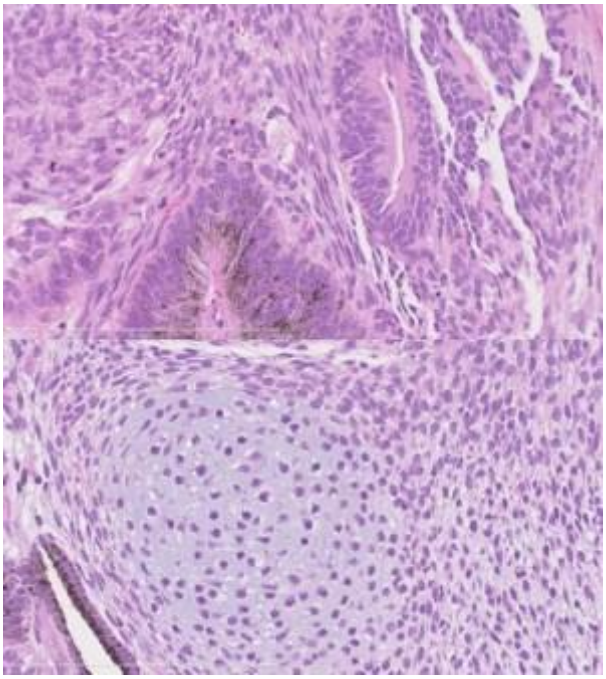
Endodermal structures were sought. They are very rare. Their observation required the use of PAS staining to visualize the mucus, observed as a small fuchsia-pink lake rather in the apical position in the cell.

Conclusion

The pluripotent iPS cells injected subcutaneously induced the formation of a subcutaneous teratocarcinoma.

The three germ layers are present, with ectoderm derivatives predominating, accompanied by mesoderm derivatives in smaller quantities. Endoderm-derived cells are very rarely observed.

Figure groupe 5 : VER_C

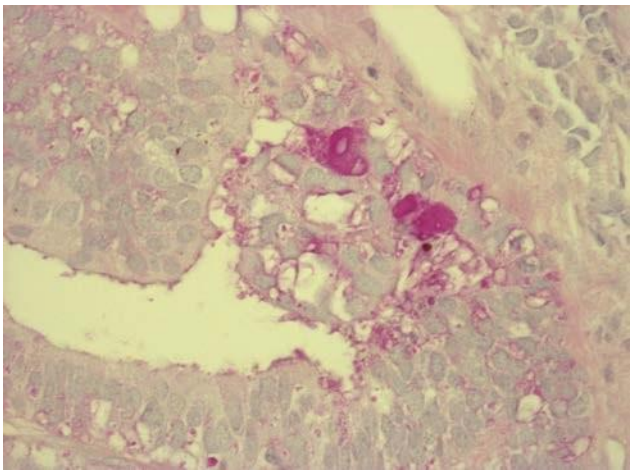


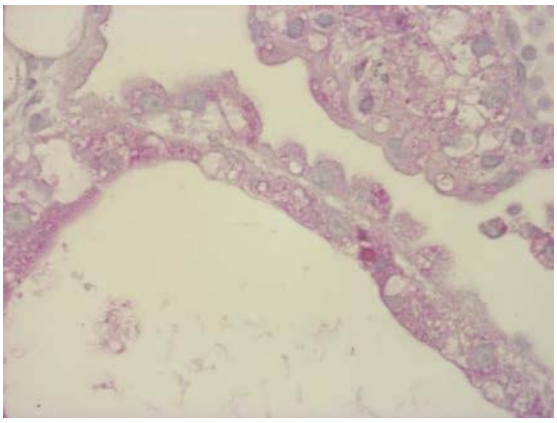
Neuroectodermal derivatives:
neural tube and melanocytes

g = 40

Mesodermal cartilage derivative

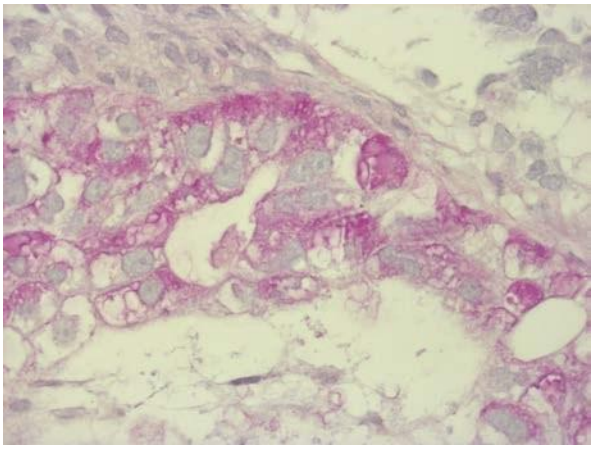
g=40





, coloration de PAS

Endodermal derivative intestinal epithelium



coloration de PAS

Group 6: CHA_P

Injection into a NOD-SCID gamma mouse, mouse F. Location: subcutaneous region on the right. Injected line: CHA_P; Number of cells injected: 3 million cells

MACROSCOPY

Observation of dense tissue nodules, measuring for mouse F = 569 mm3 => 13.5 x 9 mm2 (LxW)

MICROSCOPY

The sample consists of one or more well-defined nodules developed in the adipose tissue of the hypodermis. These nodules are variably dense with cells, sometimes with cystic areas and rare areas of tissue necrosis. Numerous embryonic structures are observed, separated by undifferentiated embryonic mesenchymal cells. The cells show signs of malignancy, with numerous mitoses noted as well as cell clusters with loss of polarity.

The majority of cells are arranged in neuroectodermal structures (neural tube primordia, sometimes accompanied by clearly pigmented melanocytes and, more rarely, neurons), associated with ectodermal structures (dental primordia +/- sebaceous glands).

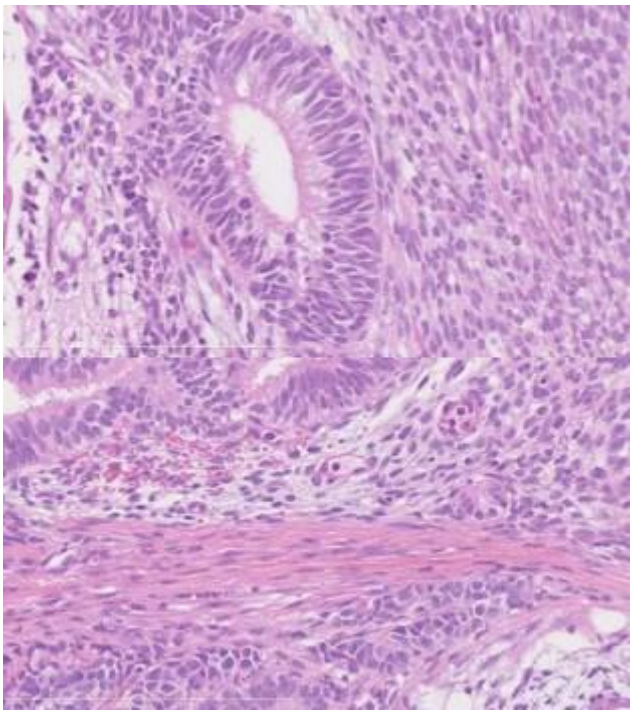
Mesodermal structures are also observed, present in smaller quantities, such as smooth muscle and, more rarely, bone.

Endodermal structures were sought. They are very rare. Their observation required the use of PAS staining to visualize mucus, observed as a small fuchsia-pink lake rather apically in the cell.

Conclusion

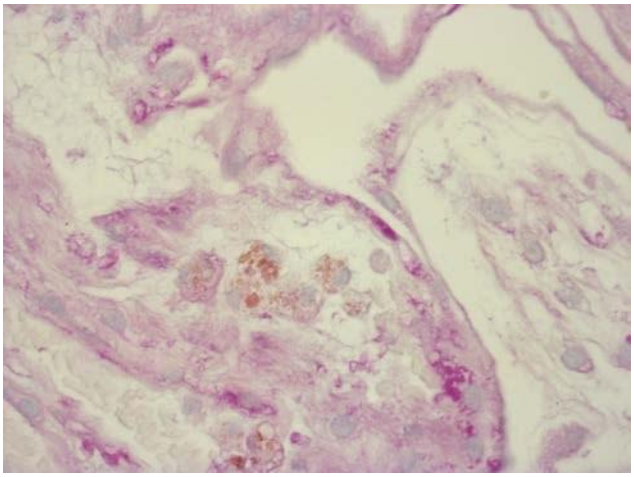
The pluripotent iPS cells injected subcutaneously induced the formation of a subcutaneous teratocarcinoma. All three germ layers are present, with ectoderm derivatives predominating, accompanied by smaller quantities of mesoderm derivatives. Endoderm-derived cells are observed only very rarely.

Figure groupe 6 : CHA_P



Dérivés neuro-ectodermiques : tube neural et mélanocytes
Boite 1507-0012, g = 40

Dérivé mésodermique cartilage
lame 1507-012, g=20



Dérivé endodermique Épithélium intestinal
lame 1507-0012 g=40
coloration de PAS