

Wt1, the mesothelium and the origins and heterogeneity of visceral fat progenitors

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One major gap in adipocyte biology has been a lack of understanding of the developmental origins of the different visceral white adipose tissue (WAT) depots and subcutaneous WAT. In a recent study we showed that most visceral WAT but no subcutaneous WAT arises from cells expressing the Wilms' tumor 1 (Wt1) gene late in mouse gestation.¹ Wt1 continues to be expressed in visceral WAT progenitors into adult life. We also showed that visceral WAT is lined by a mesothelium and provided evidence that this structure is the source of adipocytes. Our study also adds to the growing body of evidence that there is heterogeneity in the visceral progenitors, such that there are Wt1-expressing and non-expressing subsets, the relative proportions of which vary between depots. This raises the enticing prospect that the adipocytes arising from these progenitor subsets may have different properties and our preliminary data support this notion. Finally, evidence from our study and one from Spiegelman's group² suggests that Wt1 is not just a marker but regulates visceral WAT identity and the progenitor population. We discuss the implications of this work and some of the questions and future directions that arise from it.

Adipose tissues are the body's natural sites for storing excessive calories. They are composed of multiple types of cells including the mature adipocytes which make up the bulk of the tissues as well as the SVF (stromal vascular fractions, including the endothelial cells, immune

cells, preadipocytes, and adipose progenitor/stem cells). Fat can be divided into several types based on the color and location. White adipose tissues (WAT) are characterized by the presence of adipocytes with large mono-locular lipid droplets while adipocytes in the brown adipose tissues (BAT) have smaller and multi-locular lipid droplets. BATs are darker in color due to the richness in blood vessels and mitochondria content. Beige/brite adipose tissue is the newly identified category of fat.³⁻⁵ It is believed that progenitors of the beige adipose tissues normally reside in the WATs and the 'browning' process is switched on by exposure to cold. Genes and pathways that are involved in the browning process have been identified by studies reported in several elegant papers.⁶⁻⁹ Secreted and systemic factors that have been shown to induce the browning process are summarized in several recent reviews.¹⁰⁻¹²

Identifying players that are involved in adipogenesis and the browning process has provided new clues to battle obesity.¹³⁻¹⁷ Most of the adipose tissues in our body are WATs which can be further divided into the subcutaneous and the visceral fat. Individuals with central obesity, which is associated with higher risk of obesity-related diseases including type II diabetes, cardiovascular diseases and cancer, tend to have larger amounts of visceral adipose tissue (the so called 'bad fat'). On the other hand, individuals with larger amounts of subcutaneous fat tend to have better metabolic profiles and lower risks of obesity-related diseases.¹⁸ Fat pad transplantation experiment in mice suggested

that the 'good' versus 'bad' properties are intrinsic to the fat pads, and the location makes a relatively small contribution.¹⁹

Despite the knowledge that the fat pads differ in their cellular composition²⁰⁻²² and the ability to proliferate/expand in response to environmental stimulations such as high fat diet,²³ their developmental origin has remained unclear. Fat is generally regarded as coming from the mesoderm except for the depots that are in the cranial facial area which come from the neural crest²⁴ (ectodermal origin). In mice, Seale *et al* (2008) demonstrated that the *myf5*+ cells in the paraxial mesoderm give rise to the BAT and not WAT.⁷ A recent paper by Sanchez-Gurmaches *et al* showed that *myf5*+ cells can also contribute to adipocytes in retroperitoneal but not epididymal WAT.²⁵ Fat depots also differ in the timing at which they first appear in the body. In the murine system, BAT is visible as early as from E14.5²⁶ while subcutaneous/inguinal fat develops from E14-E18.²⁷

The pre-adipocytes isolated from the SVF of fat show differences in their gene expression between visceral depots as well as between visceral and subcutaneous fat.²⁸ However, Tchkonina *et al* demonstrated that pre-adipocytes from mesenteric fat had an expression profile closer to that of subcutaneous than omental preadipocytes using adult human fat tissue obtained during intra-abdominal surgery.²¹ In the study carried out by Yamamoto *et al* where adult mouse tissue was analyzed, the expression of some developmental genes (e.g. *En1*) was high in subcutaneous and perirenal WAT, but low in epididymal and mesenteric WATs.²² From the gene expression studies, it was not possible to conclude if the subcutaneous or visceral WATs have a common origin. Our work showed that, despite both being WATs, the visceral and subcutaneous adipose tissues have different developmental origins. Our study also provided evidence that the mesothelium is a novel source for the visceral adipose tissues,¹ thus tracing their origin to the lateral plate mesoderm. Identifying different origins and progenitors for visceral vs. subcutaneous WAT could lead to approaches that selectively manipulate these populations for therapy.

Wt1 Marks Adipocyte Progenitors in All Visceral Fat Depots

Our initial interest in studying adipose tissue was to understand the reason behind the rapid fat loss when we deleted *Wt1* (Wilms Tumor protein gene) ubiquitously in adult mice.²⁹ Adult mice in which *Wt1* was deleted also showed a rapid bone loss. We showed that *Wt1* expression was detected in all visceral fat depots but not in the subcutaneous WAT or the BAT. Bone and adipose tissues share the same origin, 'mesenchymal stem cells' which led us to investigate if the *Wt1*-expressing adipose cells had any characteristics of progenitors. All *Wt1*+ cells were in the SVF and not in the floating mature adipocytes. Great advances in defining and identifying stem cells in adipose tissues have been made by showing that transplanted FACS isolated cells (expressing combinations of surface markers) were able to form functional adipose depots.³⁰ Rodeheffer *et al* showed that the Lin-CD34+CD29+Sca1+CD24+ cells (hereafter called CD24+) are able to form fat pads when transplanted in lipodystrophic mice, and referred to this population of cells as the adipose stem cells.³¹ Later work from Rodeheffer's group demonstrated that the Lin-CD34+CD29+Sca1+CD24- cells (CD24-) are preadipocytes and the CD24+ population give rise to the CD24- population.³¹ Using this panel of surface markers, we showed that the majority of *Wt1*+ cells in the SVF were in the CD24- population. Some *Wt1*+ cells were also found in the CD24+ population. The proportion of the CD24+ or CD24- populations that were expressing *Wt1* differed between fat pads. Omental and heart fat had the highest percentage of cells that were expressing *Wt1*.¹

Lineage Tracing Studies Showed that *Wt1*+ Progenitors Gave Rise to Adipocytes in Visceral Fat Depots

To test if the *Wt1*+ adipose progenitors can give rise to adipocytes, we performed lineage tracing studies using the

tamoxifen inducible *Wt1*-CreERT2 transgenic line, crossed with the mTmG reporter line. As mentioned by Berry *et al*,³² we also found that the mTmG reporter line is a valuable tool for studying adipocytes. Most of the volume of any mature adipocyte is occupied by lipid droplet(s) and the nuclei are pushed to the side. The membranous nature of the fluorescent signal in the mTmG reporter line provides a useful method of marking adipocytes. In our study, labeled adipocytes were observed in the visceral adipose depots (but not subcutaneous) when tamoxifen was administered in adult mice proving that at least some adipocytes arise from *Wt1* progenitors postnatally. We were interested in knowing the developmental origin of WATs. Therefore we then administered tamoxifen in pregnant females at E14.5 and analyzed the adipose tissues in the offspring after 1.2 y. A high percentage of adipocytes was positive suggesting these adipocytes came from cells that were *Wt1* positive at E14.5. We did not observe any positive signal in the subcutaneous WAT or the BAT in this model. It is suggested that tamoxifen can be retained in the system for up to 48 hours when administered during embryonic stages.³³ We concluded that these adipocytes must have come from cells that expressed *Wt1* between E14.5-E16.5.

The Mesothelium is a Source of Visceral Fat Progenitors

We were fascinated by the physical attachment of visceral adipose depots to the organs. All visceral organs (visceral mesothelium) and the entire body cavity (parietal mesothelium) are covered by a layer of cells called the mesothelium.¹ Mesothelia have been shown to contribute to the smooth muscle cells, endothelial cells, and interstitial cells in the heart, liver, lung, and gut during development.³⁴⁻⁴⁰ In adults, the main function of mesothelia is to provide lubrication allowing movement of the visceral organs within the body cavity. They are also a known source of progenitor cells involved in scarring and neovascularization during injury.⁴¹ To our surprise, we observed

that visceral fat depots were also covered by a layer of mesothelium.¹

Wt1 is a well-known marker for mesothelia. The timing at which the mesothelia first appear and the mechanisms of mesothelia formation differ for each organ. In the E9.5 heart in mice, cells in the proepicardium migrate and form a sheet of cells which then cover the surface of the heart forming the epicardium (mesothelium of the heart⁴²). In the intestine and lung, recent work by Bader and colleagues showed that the progenitors of mesothelium that covers the lung and gut reside in the respiratory and alimentary tracts (these lack an equivalent to the proepicardium structure seen for epicardium formation).⁴³

We hypothesized that mesothelia might be a source of visceral fat and this would explain the reason why only visceral depots were positive for adipocytes that came from Wt1-expressing cells. To test this, we cultured the epididymal appendage of the newborn pups from the tamoxifen-inducible Wt1-lineage tracing model. The epididymal appendage from the newborn pups is a thin translucent sheet of cells which will later form the epididymal fat. Cells with lipid droplets in the epididymal fat are not visible till postnatal day 4–7.⁴⁴ Whole mount staining of the epididymal appendage showed that it was covered by mesothelia. We cultured this sheet in matrigel supplemented with adipogenesis media and 4-OH tamoxifen and demonstrated that Wt1-lineaged cells in this sheet were capable of making adipocytes. In addition, we showed that Wt1+ cells in this sheet expressed high levels of mesothelial markers such as mesothelin and UPK3B. We also performed a short lineage tracing experiment by dosing the female with tamoxifen at E14.5 and analyzed the embryos at E16.5 and showed that mesothelia that covered the surface of visceral organs were labeled.

We also determined whether the mesothelial cells expressed any of the adipose progenitor markers. The panel of adipose stem cell markers is based on work characterized in adipose tissues and had not previously been functionally tested in other tissues. Our result showed that most adult and postnatal day 1 mesothelial cells were in the Lin-CD31-CD34+CD29+

Sca1+CD24- populations. Mesothelial cells from earlier developmental stages showed a similar but not identical pattern of expression. At E14.5, most mesothelial cells were in the Lin-CD31-CD34+CD29+Sca1- but CD24+ population. The mesothelial cells lost CD24 expression and gained expression of Sca1 rapidly as development progressed, a similar scenario to that observed for fat progenitors in the SVF³¹ This demonstrated that the gene expression pattern of mesothelia was not in a fixed state but it was dynamic and underwent rapid changes. Analyzing the exact genetic blueprint of mesothelia at specific developmental stages, ages, variation between organs, and their functional outputs is likely to give new insights into pathways involved in adipogenesis and how these differ from other mesothelia-associated tissues.

Although we have provided multiple evidence suggesting mesothelia could be a potential visceral WAT source including showing that 1) visceral WAT has a mesothelial layer and Wt1 is one of the most commonly used markers for studying mesothelia, 2) Wt1+ cells in the epididymal appendage expressed high levels of mesothelia-enriched genes, 3) only mesothelia were labeled in the short tamoxifen pulsing experiment (E14.5-E16.5), and 4) mesothelial cells expressed adipose progenitor markers, we would still like to strengthen this result by using a mesothelial-specific cre (e.g., the mesothelin-Cre⁴¹) for cell fate analysis *in vivo*. However, in support of our hypothesis, a recent study using lineage tracing showed that embryonic epicardial progenitors were able to contribute to epicardial fat in postnatal 8 weeks old mice. This was achieved by injecting an adenovirus that expresses mesothelin (Msln) Cre in the E12.5 Rosa26mTmG/+ embryonic heart.⁴⁵

Adipose tissue is often seen to be characterized by ill-defined boundaries. However, we noticed that visceral adipose tissues can still be separated into discrete depots even in obese models in rodents. We propose that the mesothelia covering the surface of visceral adipose depots help to keep the depots in shape as well as to dictate where fat is formed. The adipose mesothelia may perform the same functions as mesothelia have in other organs

(e.g. paracrine effects and producing mesenchymal progenitors through an epithelial to mesenchyme transition or more correctly a mesothelial to mesenchyme transition). Given this and the fact that each fat depot has a different composition of cells and this is reflected by their distinctive transcriptomic profiles, it is tempting to consider the depots as little ‘mini-organs’ with different ‘flavors’ and functions.⁴⁶

Heterogeneity of Visceral WAT Progenitors

Our lineage data and FACS analysis suggested that not all visceral WAT progenitors express Wt1 and the proportion of Wt1+ progenitors varies between depots. Preliminary data in our study also suggested that the adipocytes that arose from Wt1+ progenitors differed in their physical properties (size and number of droplet/cell) from the adipocytes that were derived from Wt1-negative progenitors. The chemical, transcriptomic, metabolomic properties, and turnover rate of the adipocytes arising from these 2 progenitors await to be revealed.

The turnover rate of adipocytes is not totally clear (estimates vary from 1–5% per day in mice⁴⁷ or a 10% turnover of adipocytes per year in human⁴⁸). The fact that we saw adipocytes that were lineage traced from E14.5 after 1.2 y suggested that either the cells that were positive for Wt1 at E14.5 had the ability to self-renew or the adipocytes that were highlighted had very slow (or no) turnover, or both. Future studies determining the half-life and turnover rate of adipocytes are of great importance.

A cartoon illustration of the origin and heterogeneity of adipose tissue is summarized in **Figure 1**. In an E8.5 mouse embryo, Wt1 is first expressed in the intermediate mesoderm and lateral plate mesoderm (highlighted in green). At E14.5, the Wt1-expressing mesothelia, derived from the lateral plate mesoderm, were shown to be one possible source of mature adipocytes in all visceral fat pads. The myf5-positive population of cells in the paraxial mesoderm have been shown to contribute to BAT as well as retroperitoneal WAT, adding to the

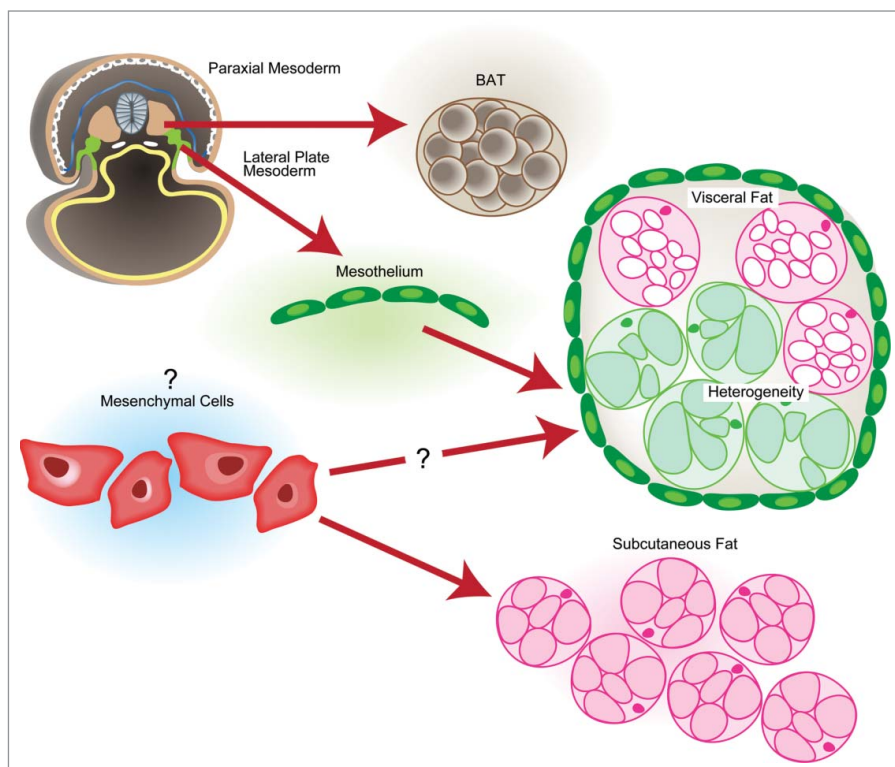


Figure 1. A simplified cartoon placing our findings (Reference 1) into the context of pre-existing knowledge on the origins of the major murine adipose tissues. In an E8.5 mouse embryo, the *Myf5*⁺ cells in the paraxial mesoderm give rise to BAT. Mesothelial cells (express *Wt1*, indicated in green), which come from the lateral plate mesoderm, were able to contribute to adipocytes in all visceral fat pads (indicated in green). Like other visceral organs, visceral fat pads are also covered by mesothelium. The origin of subcutaneous WAT is not clear.

heterogeneity of visceral WAT. The exact developmental origin of the subcutaneous WAT is not known although it is often to be described ambiguously as coming from ‘mesenchymal cells.’

Wt1 was first associated with adipose tissue when we deleted *Wt1* ubiquitously and the mutant mice had a fat loss phenotype.²⁹ The visceral only expression and lineage result provoked us to explore the developmental origin of fat. The next stage will be to investigate the function of *Wt1* in regulating adipogenesis. We showed that *Wt1* might be important for the growth of adipose progenitors. Deleting *Wt1* in adipose depots led to a reduction of adipose progenitors in some depots.¹ Encouragingly, Spiegelman and colleagues showed that the thermogenic and inflammation pathways were switched on when *Wt1* was deleted using an adiponectin-Cre.² In addition, in support of our mesothelial hypothesis, 2 genes that ranked top

among the visceral progenitor markers in their paper are mesothelial genes (mesothelin and uroplakin). This brings *Wt1* to the center stage of visceral adipogenesis. We have identified a population of adipose progenitors that were only expressed in the visceral adipose depots. This provides a useful tool in answering some key questions in this field including the heterogeneity of progenitors and adipocytes that are derived from them.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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