Functional validation of preadipocytes isolated from adipose tissue of CD248 WT and KO mice to investigate insulin signalling pathways

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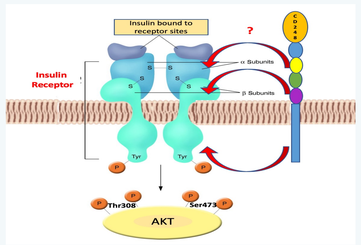
BACKGROUND

CD248 is a pro-inflammatory transmembrane glycoprotein that participates in different biological pathways. It is expressed at low levels in stromal and perivascular cells and highly expressed in preadipocytes and mature adipocytes of white adipose tissue. Lack of CD248 in mice protects against diet induced obesity, insulin resistance and glucose intolerance.



Delineating the mechanisms by which CD248 functions in glucometabolism would be aided by having primary preadipocytes from the genetically modified mice. There are different methods for isolating preadipocytes:

1. Explant culture

This technique allows small fragments of tissue to adhere to the growth surface, which may give rise to an outgrowth of cells,. It is causes minimal damage to the cells but the yield may be limiting.

2. Enzymatic digestion

This is a common method for isolating cells with collagenase, but the process may damage the natural microenvironment of adipose tissue, and may affect viability, phenotype, and differentiation potential.

3. Magnetic labelling isolation

In this method adipose tissue derived progenitor cells are isolated by depletion of non-target cells followed by positive selection of the target cells.

OBJECTIVE

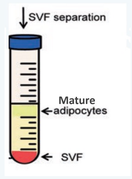
To validate that isolated primary preadipocytes retain their properties *ex vivo*, we will investigate the insulin signalling pathways in primary preadipocytes from white adipose tissue of CD248 WT and KO mice by quantifying insulin-triggered phosphorylation of AKT by Western blot.

METHODS

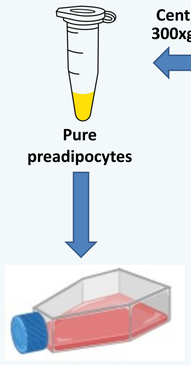
Excise adipose tissue from euthanized mice and cut into small pieces with 2 sterile sharp blades

Digest with collagenase for 30-40 min 37˚C shaking water bath

Filter through 100 and 70um nylon mesh and centrifuge 500xg 15min and 5min



Remove the supernatant and resuspend the pellet of SVF cells in MACS buffer



Centrifuge 300xg/5min

MS column

4˚C /15min/dark

Add the Non-Adipocytes Progenitor Depletion Cocktail to the resuspended cells

+

4˚C /15min/dark

LS column

Centrifuge 300xg/5min

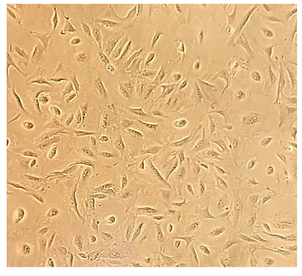
+

Add adipocytes Progenitor Isolation Cocktail to the resuspended cells

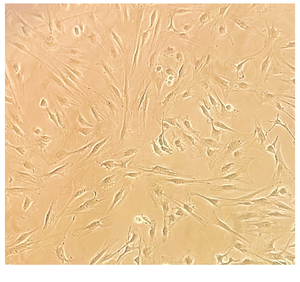
SVF

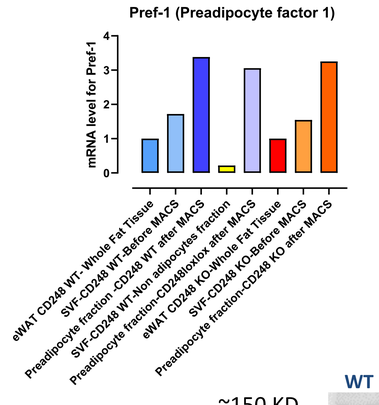
RESULTS

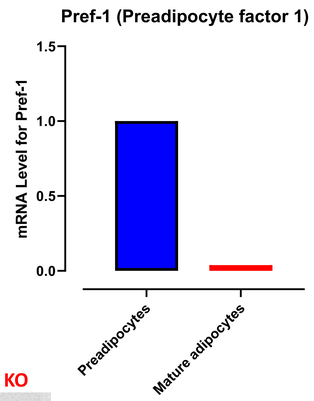
CD248 WT Preadipocytes

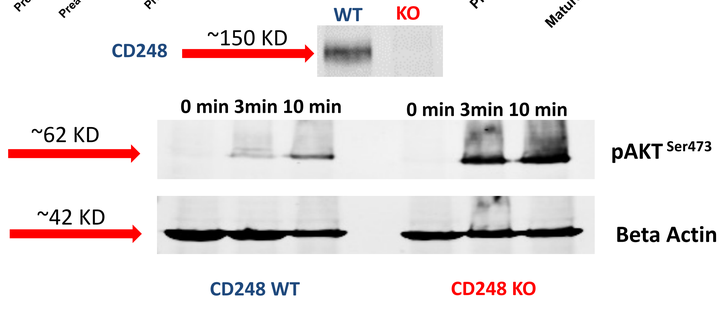


CD248 KO Preadipocytes









Preadipocytes-stimulated with insulin at different time points

CONCLUSIONS

1. Purified preadipocytes isolated from the CD248 WT & KO mice revealed increased sensitivity of the KO cells to insulin triggered activation of the PI3K/AKT pathway. The findings are in line with *in vivo* observations of increased resistance of CD248 KO mice to high fat diet induced diabetes and obesity.

2. Having a validated technique to isolate and culture pure preadipocytes that reflects *in vivo* findings, will facilitate investigations of the molecular mechanisms and functional interactions between CD248 and the insulin signalling pathway components.

FUTURE DIRECTIONS

Isolate and expand preadipocytes from other fat depots of CD28 WT and KO mice to investigate the mechanisms by which CD248 modulates glucometabolism.

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