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METHODS PAPER

Aldehyde dehydrogenase activity of Wharton jelly mesenchymal stromal cells: isolation and characterization

Mehdi Najar · Emerence Crompot [ID] · Leo A. van Grunsven · Laurent Dollé · Laurence Lagneaux

Abstract Mesenchymal stromal cells (MSCs) are promising tools in regenerative medicine and targeted therapies. Although different origins have been described, there is still huge need to find a valuable source harboring specific subpopulations of MSCs with precise therapeutic functions. Here, we isolated by fluorescence activated cell sorting technique, two populations of Wharton's jelly (WJ)-MSCs based on their aldehyde dehydrogenase (ALDH) activity. Two different ALDH activities (low vs. high) were thus observed. We then analyzed their gene expression profile for stemness, phenotype, response to hypoxia, angiogenesis, hematopoietic support, immunomodulation and multilineage differentiation abilities (osteogenesis, adipogenesis, and chondrogenesis). According to ALDH activity, many differences in the mRNA expression of these populations were noticed. In conclusion, we provide evidences that WJ harbors two distinct populations of MSCs with different ALDH activity. These populations seem to display specific functional competences that may be interesting for concise therapeutic applications.

Introduction

Mesenchymal stromal cells (MSCs) are attractive therapeutic tools since their potential use in cellular immunotherapy and regenerative medicine (Forostyak et al. 2013; Dunavin et al. 2017; Zimmerlin et al. 2013). Finding therapeutic valuable sources of MSCs is a major concern for the field. Several sources have been thus reported such bone marrow (BM) (Najar et al. 2015), adipose tissue (AT) (Busser et al. 2015), foreskin (Najar et al. 2016a) and dental pulp (Kang et al. 2016) with contrasting results. Embryonic tissues like placenta (Lee et al. 2012) and umbilical cord (CB) (Rizk et al. 2017) seem to be important alternatives. The gelatin that surrounds the umbilical cord vessels called Wharton's jelly (WJ) is very promising; indeed, the WJ-MSCs have been discovered to be an ideal source of stromal cells (Beeravolu et al. 2017). Indeed, these cells have already presented high capacity to promote tissue regeneration and repair (Kamolz et al.

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2014) as well as demonstrated great immunotherapeutic features (Nagamura-Inoue and He 2014). Improving cell therapy implies also the research of specific populations with concise functional competences that may be interesting for targeted therapeutic applications. The aim of this work was to find a suitable method for isolating sub-populations of MSCs based on aldehyde dehydrogenase (ALDH) activity. Bodipy-aminoacetaldehyde (BAAA) contained in Aldefluor® assay (Storms et al. 1999; Alison et al. 2010) allows detecting cells with ALDH activity. ALDH enzymes were reported to have critical roles in cell protection, proliferation and differentiation (Balber 2011). The use of ALDH activity was thus referred as a reliable marker to isolate stem or progenitor cells (Ma and Allan 2011).

We first used fluorescence activated cell sorting (FACS) for isolating WJ-MSCs based on their ALDH activity (Herzenberg et al. 2002). Based on their ALDH activity, two populations of MSCs (referred as ALDH$^+$ and ALDH$^-$) were obtained and analyzed for their transcriptome. Accordingly, we noted several differences in their gene expression profile for stemness, cell cycle, immunomodulation, hypoxia response faculty, phenotype, hematopoietic support and multilineage capacities. Collectively, by selecting sub populations of WJ-MSCs with specific gene profile, we may improve cell therapy by delivering targeted therapeutic strategies.

Materials and methods

Ethics

This study was approved by the Bordet Institute Ethics Committee (Belgium) and conducted in accordance with the Declaration of Helsinki (1964). WJ samples were obtained following written informed consent from mothers (n = 4).

Isolation and culture of WJ-MSCs

The WJ were obtained after the full-term delivery and were directly processed. All the procedures for the isolation, culture and characterization of WJ were previously described (Najar et al. 2010a). Briefly, cords were transferred to an aseptic saline buffer, washed with HBSS (Lonza) and cut in small pieces.

The samples were sectioned longitudinally to expose the WJ on the surface and the matrix was completely immerged with Dulbecco's Modified Eagle's Medium with low glucose (Lonza: DMEM-LG) supplemented with 10% FBS (Sigma-Aldrich), 2 mmol/L L-glutamine and 50 U/mL penicillin/streptomycin (Lonza) during 15 days. Migratory and adhesive properties allow the WJs to exit and attach to the plastic surface. After this time, umbilical cord fragments were removed from the well to allow cell culture. When sub-confluence (80–90%) was achieved, adherent cells were harvested by TrypLE Select (Gibco, LifeTechnologies) and expanded until the desired passage.

Characterization of WJ-MSCs

The phenotype of MSCs was established by flow cytometry (MACSQuant®, Miltenyi Biotec, Netherlands) using the following monoclonal antibodies: anti-CD45-FITC and anti-HLA-DR-PE (Exalpha Biologicals, Maynard, MA), anti-CD34-PE and anti-CD73-PE (BD Biosciences, San Diego, CA, USA), anti-CD14-PE, anti-CD19-PE, anti-CD105-FITC and anti-CD90-PE (R&D systems, Minneapolis, MN, USA). The trilineage potential of MSCs was confirmed through inducing differentiation into adipogenic, osteogenic and chondrogenic lineages using the appropriate culture conditions (NH media, Miltenyi Biotec).

Fluorescence activated cell sorting (FACS) analysis

Cell sorting was conducted using a FACS-Aria (BD Biosciences, San Jose, CA) as previously shown earlier (Mandelli et al. 1999). In summary, the instrument quality control was checked using Cytometer setting and tracking beads (CS&T) and software (FACSDiva) on a daily basis throughout this study. To analyze live cells and excluding debris as well as residual erythrocytes, two gates were respectively placed for scatter population and propidium iodide (PI) (Fluka, 70335). We also incorporated a doublet discriminating gate based upon height versus area of the side scatter signals. These settings were then adopted during the entire assay and the analysis was done using the Flo-Jo program (Tree Star, Ashland, www.flojo.com).

ALDH-positive and -negative cell population
detection and sorting

The Aldefluor kit is used to isolate a population with
high ALDH versus low ALDH enzymatic activity
according to the manufacturer's instructions (Stem-
Cell Technologies). Fluorescent ALDH substrate
(BODIPY®—aminoacetaldehyde—BAAA/Aldeflu-
or®) can be used to identify and isolate ALDH⁺ cells
by flow cytometry according to our previous works
(Dolle et al. 2012, 2015). Dissociated single cells (with
trypsin) were suspended in Aldefluor assay buffer
containing the BAAA (1 μM per 3 × 106 cells) and
incubated at + 37 °C without agitation during 50 min.
BAAA (a non-polar fluorescent molecule) is taken up
by viable cells through passive diffusion. The BAAA
is composed of the bodipy molecule (which contains
the green fluorescence) and the amino-acetaldehyde
(which is the substrate for ALDH1A1). BAAA is
metabolized by one of ALDH isoform (Moreb et al.
2012) to a carboxylate ion BAA⁻ which is retained
intracellularly. By becoming fluorescent, BAA-allows
cells with high levels of ALDH to be isolated by
FACS. The sorting gate of the ALDH⁺ cells was
established using DEAB-treated cells as a reference.
For all subsequent procedures, samples were con-
stantly maintained at + 4 °C to prevent efflux.
Aldefluor fluorescence was excited at 488 nm, and
fluorescence emission was detected using a standard
fluorescein-isothiocyanate 530/30 nm band-pass fil-
ter. All samples were thus sorted into 2 different
collector tubes, namely "ALDH⁺" and "ALDH⁻".

Gene expression analysis by Real Time PCR
(qPCR)

After the sorting, the cells were directly spin-down by
centrifugation. The extraction of the total mRNA was
performed by mixing the cell lysate buffer [10 mL of
BL-buffer from Promega (ref Z103c) with 10 μL of
thioglycerol (ref A208B)]. We used the RNeasy micro
kit (ref 74004) from Qiagen to ensure optimal RNA
purification because the number of cells obtained after
the cell sorting was roughly small for some samples.
The reverse transcription reaction was performed with
1 mg RNA using qScript cDNA SuperMix (Quanta
Biosciences). Transcripts were quantified by qRT-
PCR using 10 ng of cDNA, SYBR Green PCR Master
Mix (Applied Biosystems, Lennik, Belgium) and

0.32 mM forward and reverse primers. The primers
(Table 1) were designed thanks to ProbeFinder online
software (Roche) or Primer Express 2.0 software
(Applied Biosystems). The ABI Prism 7900 HT
system (Applied Biosystems) was used to carry out
the reactions and GAPDH gene used as a housekeep-
ing gene to quantify and normalize the results.
Dissociation curves were generated and the specificity
of the PCR reactions was confirmed. The comparative
$\Delta\Delta Ct$ method was finally used for the data analysis;
data were normalized with the GAPDH genes to
obtain the ΔCt and were after calibrated with the
geometric mean of the GAPDH ΔCt to generate the
$\Delta\Delta Ct$. Fold changes were then calculated as fold
change = $2^{-\Delta\Delta Ct}$.

Statistical analysis

Data are presented as mean ± standard error of the
mean (SEM). The matched paired t test was used to
compare the both sorted fractions. All analyses were
performed with GraphPad Prism version 5.00 for
windows (GraphPad Software, www.graphpad.com).

Results

ALDH activity dependent sorting and isolation
of WJ-MSCs

Following the sorting, 2 populations with high and low
ALDH enzymatic activity were thus isolated (ALDH⁺
and ALDH⁻). ALDH activity was significantly dif-
ferent in these 2 populations: 0.9 ± 0.04 UA for the
ALDH⁻ and 36.5 ± 1.36 UA for the ALDH⁺ popu-
lation, $p = 0.0001$ (Fig. 1).

Gene expression profiling and analysis of ALDH
sorted WJ-MSCs populations

*Figure 2: Stemness (Nanog; Octamer-binding
transcription factor 4(Oct4); Rex1)*

Gene expression related to the stemness properties
showed differences according to ALDH activity. We
observed a significant increase in ALDH⁺ cells for the
three targets compared to ALDH⁻ population. We
obtained in ALDH⁻ cells an expression of
2788 ± 42.3, 450.3 ± 19.7, 215.8 ± 8.9 versus

Table 1 qRT-PCR primers

Transcripts	Forward	Reverse
Cell cycle		
p53	AGGCCTTGGAACTCAAGGAT	CCCTTTTTGGACTTCAGGTG
p21	CGAAGTCAGTTCCTTGTGGAG	CATGGGTTCTGACGGACAT
p16	TGCCTTTTCACTGTGTTGGA	TGCTTGTCATGAAGTCGACAG
pRB	TCCTGAGGAGGACCCAGAG	AGGTTCTTCTGTTTCTTCAAACTCA
CDC25A	CGTCATGAGAACTACAAACCTTGA	TCTGGTCTCTTCAACACTGACC
FosB	CCGAGAGGAGACGCTCAC	CTGCTGCTAGTTTATTTCGTTCC
STAT1	GACTGAGTTGATTTCTGTGTCTGAA	ACACCTCGTCAAACTCCTCAG
CCNA	GGTACTGGAGTCCGGGAACC	GAAGATCCTTAAGGGGTGCAA
CCNB	CCTCCGGTGTTCTGCTTC	TTCAGCATTAATTTTCGAGTTCC
CCNE	CTTCACAGGGAGACCTTTTAC	CATTCAGCCAGGACACAATAG
CDK1	TGGATCTGAAGAAATACTTGGATTCTA	CAATCCCCTGTAGGATTTGG
CDK2	GCTAGCAGACTTTGGACTAGCCAG	AGCTCGGTACCACAGGGTCA
Hypoxia		
HIF1α	TGGAATGGAGCAAAAGACAA	CAGCTGTGGTAATCCACTTTCA
HIF2α	CATCATGCGACTGGCAAT	GCTTCGGACTCGTTTCAGA
GLUT1	CTTCCTACCCAACCACTCAAA	CCCTCTCCTCCCTGCACT
Stemness		
SOX2	CCATCCACACTCACGCAAAA	CCCCCAAAAAGAAGTCCCAA
REX1	CCCTGGACTGCGAGATGT	AGGCTTCACGAAGGTGTCAT
NANOG	ACAACTGGCCGAAGAATAGCA	GGTTCCCAGTCGGGTTCAC
OCT4	CTTCGCAAGCCCTCATTTCAC	TTGATGTCCTGGGACTCCTCC
Angiogenesis		
ANG1	TTCCTGTCAAGTCATCTTGTGAA	TTTTATTAAGGTTGCACATCCAAG
ANG2	GGCAGCGTTGATTTTCAGAG	TTGCGAAACAAACTCATTTCC
FLT1	ACATTGGCCACCATCTGAAC	GCAGTATTCAACAATCACCATCA
VEGF	CTACCTCCACCATGCCAAGT	GCAGTAGCTGCGCTGATAGA
Phenotype		
CD54	AGTGATCAGGGTCCTGCAA	GGGAGGGAGTCCTCCAATAC
CD58	CCAATGCATGATACCAGAGCAT	CCAATGCATGATACCAGAGCAT
CD106	GGCTGTGAATCCCCATCTT	AATTGGTCCCCTCACTCCTC
CD146	GGGTACCCATTCCTCAAGT	CAGTCTGGGACGACTGAATG
CD200	TCTACCTACAGCCTGGTTTGG	TGGGTCACCACTTGCACTT
Immuno		
GAL1	AAGCTGCCAGATGGATACGAA	CGTCAGCTGCCATGTAGTTGA
COX1	CCTGCAGCTGAAATTTGACCCA	ACCTTGAAGGAGTCAGGCATG
COX2	GCTCAAACATGATGTTTGCATTC	GCTGGCCCTCGCTTATGA
HGF	CAATGCCCTCTGGTTCCCTT	AGGCAAAAAGCTGTGTTCGTG
LIF	TGAAAACTGCCGGCATCTGA	CTGTGTACTGCCGCCAAGA
Hematopoiesis		
IL-6	AAATTCGGTACATCCTCGACGG	GGAAGGTTCAGGTTGTTTTCTGC
IL-8	CTGTTAAATCTGGCAACCCTAGTCT	CAAGGCACAGTGGAACAAGGA
SCF	AGCCAGCTCCCTTAGGAATG	CGAGTGGGTCTAGCGGAAAG
MMP2	TGATCTTGACCAGAATACCATCGA	GGCTTGCGAGGGAAGAAGTT
SDF1	CTGTGCCCTTCAGATTGTAGCC	CTGTAAGGGTTCCTCAGGCG

Table 1 continued

Transcripts	Forward	Reverse
Osteogenesis		
OSX	TAGGCAGCAGCAGTAGCAGA	TCTGACTCCAGAGTCCTTGCT
BSP	TACACGGGCGTCAATGAATA	AGGTTCCCCGTTCTCACTTT
RUNX2	TTACTTACACCCCGCCAGTC	TATGGAGTGCTGCTGGTCTG
OPN	TTGCAGTGATTTGCTTTTGC	GCCACAGCATCTGGGTATTT
OPG	GGCAACACAGCTCACAAGAA	CGCTGTTTTCACAGAGGTCA
Adipogenesis		
AdipoQ	GCTCTGTGCTCCTGCATCTG	GAGTCCATTACGCTCTCCTCC
PPARγ	CACAAGAACAGATCCAGTGGTTGCAG	AATAATAAGGTGGAGATGCAGGCTCC
KLF2	CATCTGAAGGCGCATCTG	CGTGTGCTTTCGGTAGTGG
KLF5	GGCTTTACTCAAGCAGATCTCATC	CCCTACCCATGTTGAGACG
CEBPδ	TGACAGCCTCGCTTGGACG	CTCTCGTCGTCGTACATGGC
CEBPα	TATAGGCTGGGCTTCCCCTT	AGCTTTCTGGTGTGACTCGG
Chondrogenesis		
COL2α1	CCCATCTGCCCAACTGACC	CCAGTCCGTCCTCTTTCACC
ACAN	GTGAGGAGGACATCACCGTC	AAGGCAGTGGCCCCTATTTC
SOX9	GTACCCGACACTTGCACAAC	TCGCTCTCGTTCAGAAGTCTC
COMP	CAGGGAGATCACGTTCCTGA	GGCCGGTGCGTACTGAC
Housekeeping		
GAPDH	AATCCCATCACCATCTTCCA	TGGACTCCACGACGTACTCA

5184 ± 364.3, 1272 ± 57.7, 354.9 ± 14.15 in ALDH$^+$ cells for Rex1, Nanog and Oct4 respectively ($p = 0.0084$, $p = 0.0018$, $p = 0.0014$) (Fig. 2).

Figure 3: Proliferation/cell cycle (CyclinA (CCNA), CCNB, CCNE; CDK1, CDK2, Fos proto-oncogene (Fosb); p21; p53; p16; retinoblastoma protein (pRB); cell division cycle 25A (CDC25A); signal transducer and activator of transcription (STAT1))

Distinct gene expressions concerning proliferation and cell cycle were observed in both populations according to ALDH activity. We observed a significant increase in ALDH$^+$ cells for all the targets compared to ALDH$^-$ population. About the cycline dependent kinase inhibitors, we obtained in ALDH$^-$ cells an expression of 21,491 ± 325, 105,959 ± 5793, 15,335 ± 638.3, versus 73,235 ± 4697, 152,135 ± 4209, 32,872 ± 1555 in ALDH$^+$ cells for p16, p21 and p53 respectively ($p = 0.0014$, $p = 0.0075$, $p = 0.0034$). We also noted increased expressions for two cyclin

dependent kinase CDK1 and CDK2 with significant p value (19,271 ± 396.5, 1810 ± 129 versus 23,174 ± 1078, 4033 ± 334.8). The mRNA of FosB in WJ-MSCs was 2933 ± 165 in ALDH$^-$ and 8141 ± 546.6 in ALDH$^+$ cells ($p = 0.0029$). CCNA, CCNB and CCNE presented same profile with 1086 ± 27.86, 182.2 ± 13.92, 867.5 ± 34.83 versus 1478 ± 63.8, 287.7 ± 8.84, 1381 ± 75.4 ($p = 0.0023$, $p = 0.0007$, $p = 0.0065$). Finally, PrB, Stat1 and CDC25A were also more expressed in ALDH$^+$ cells compared to ALDH$^-$ cells with significant p values ($p = 0.0410$, $p = 0.0053$, $p = 0.0002$) (Fig. 3).

Figure 4: Phenotype (melanoma cell adhesion molecule CD146 (MCAM); CD200; vascular cell adhesion molecule 1 CD106 (VCAM-1); intercellular adhesion molecule 1 CD54 (ICAM-1); CD58 (LFA-3))

The genes related to the phenotype of MSCs significantly differed between ALDH$^+$ and ALDH$^-$ subsets of WJ-MSCs. CD146 was the most expressed with
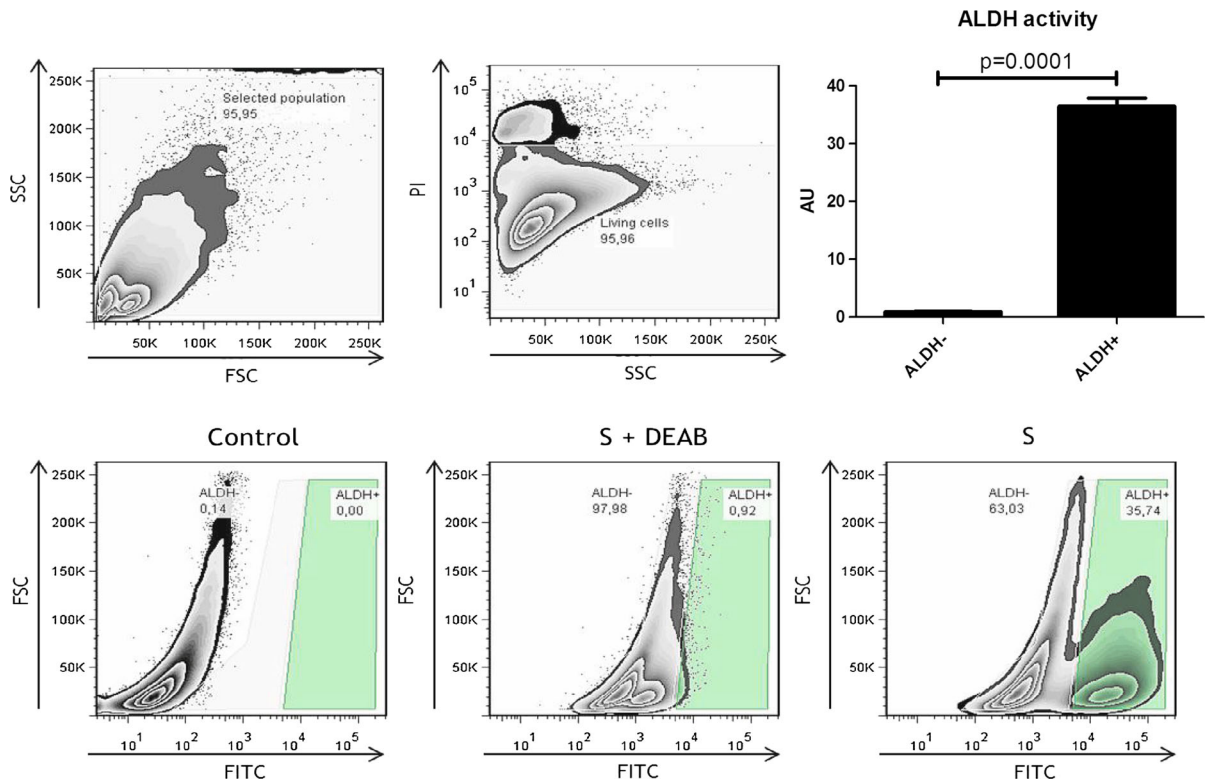
Fig. 1 Identification and sorting of WJ-MSCs based on ALDH activity. Representative pictures showing the flow cytometry profile of ALDH$^+$ and ALDH$^-$ populations of WJ-MSCs based on their respective ALDH activity. ALDH activity within WJ-MSCs are reported as the mean $\pm$ SEM (Arbitrary unit) (S for substrate)
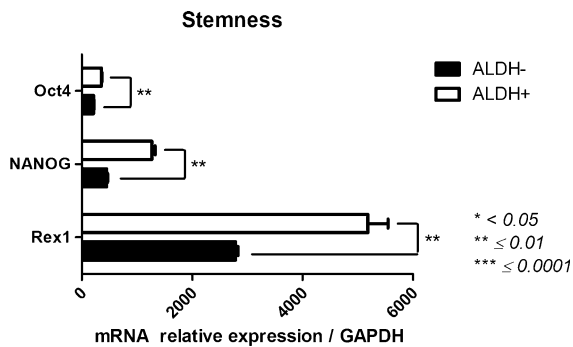

Fig. 2 Stemness profile of ALDH$^+$ and ALDH$^-$ WJ-MSCs. After sorting ALDH$^+$ and ALDH$^-$ WJ-MSCs by FACS technique, we determined by qPCR their expression of *Oct4*, *Nanog* and *Rex1* as description of their stemness. Data are presented as mean $\pm$ SEM of mRNA gene expression relative to *GAPDH* expression

62,344 $\pm$ 4217 in ALDH$^+$ cells and 30,557 $\pm$ 1987 in ALDH$^-$ cells ($p = 0.0133$). CD54, CD58 and CD106 presented the same profile with significant p values ($p = 0.0044$, $p = 0.0009$, $p = 0.0014$).

CD200 was less expressed in ALDH$^-$ population (23,065 $\pm$ 2233 vs. 42,478 $\pm$ 2082, $p = 0.0108$) (Fig. 4).

Figure 5: Hypoxia response (hypoxia-inducible factor (HIF)-1α; HIF-2α; Solute carrier family 2 member 1 (GLUT1))

Hypoxia responses genes were distinctively expressed between both ALDH populations. We clearly observed three significant increases for HIF1α, HIF2α and GLUT1 in ALDH$^+$ cells ($p = 0.0174$, $p = 0.0016$, $p = 0.0043$). We noticed that GLUT1 was the most expressed with mean of 55,813 $\pm$ 4288 in ALDH$^-$ population versus 80,907 $\pm$ 2631 in ALDH$^+$ cells (Fig. 5).
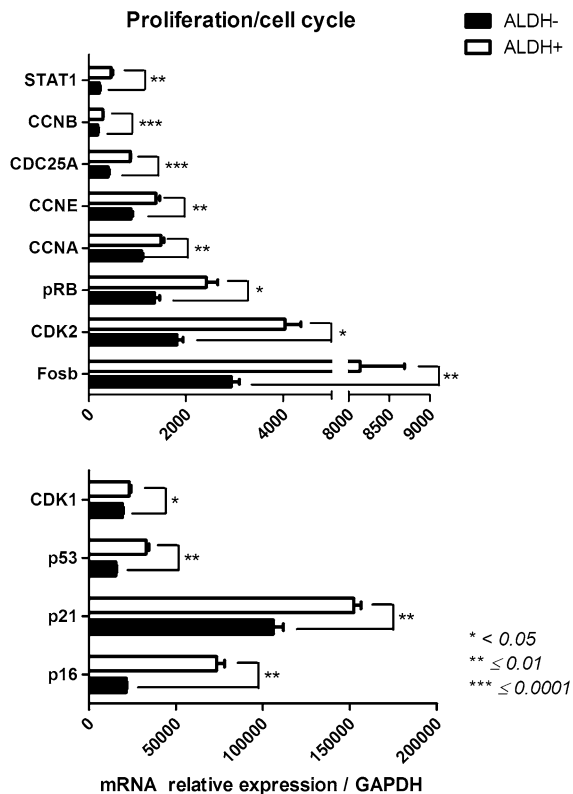
Fig. 3 Proliferation and cell cycle profile of ALDH⁺ and ALDH⁻ WJ-MSCs. After sorting ALDH⁺ and ALDH⁻ WJ-MSCs by FACS technique, we evaluated by qPCR their expression of *FosB*, *CDK1*, *CDK2*, *Cyclin A*, *Cyclin B*, *Cyclin E*, *p16*, *p21*, *p53*, *pRB* and *STAT1* as indication of their proliferation and cell cycle. Data are presented as mean ± SEM of mRNA gene expression relative to *GAPDH* expression
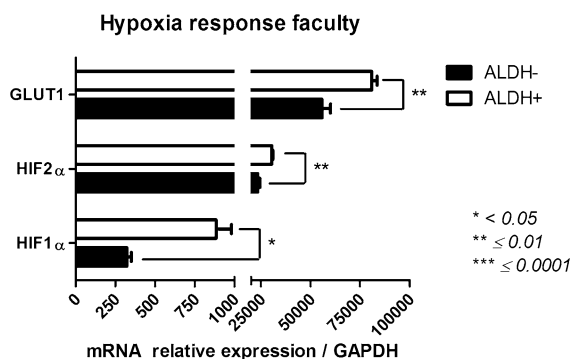

Fig. 4 Hypoxia response faculty of ALDH⁺ and ALDH⁻ WJ-MSCs. After sorting ALDH⁺ and ALDH⁻ WJ-MSCs by FACS technique, we investigated by qPCR their expression of *HIF-1α*, *HIF-2α*, *Glut1* as proof of their hypoxia response faculty. Data are presented as mean ± SEM of mRNA gene expression relative to *GAPDH* expression
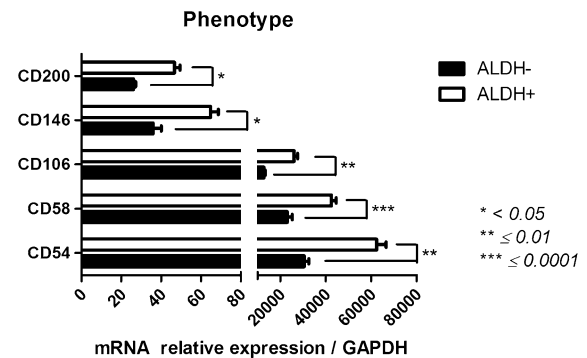

Fig. 5 Phenotype of ALDH⁺ and ALDH⁻ WJ-MSCs. After sorting ALDH⁺ and ALDH⁻ WJ-MSCs by FACS technique, we assessed by qPCR their expression of *CD146*, *CD200*, *CD106*, *CD54*, and *CD58* as characterization of their phenotype. Data are presented as mean ± SEM of mRNA gene expression relative to *GAPDH* expression
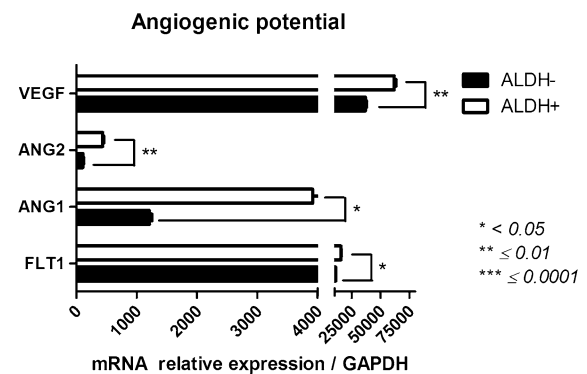

Fig. 6 Angiogenic potential of ALDH⁺ and ALDH⁻ WJ-MSCs. After sorting ALDH⁺ and ALDH⁻ WJ-MSCs by FACS technique, we studied by qPCR their expression of *ANG1*, *ANG2*, *FLT1* and *VEGF*, as evidence of their angiogenic potential. Data are presented as mean ± SEM of mRNA gene expression relative to *GAPDH* expression

Figure 6: Angiogenesis (angiopoietin (ANG)1, ANG2, Fms related tyrosine kinase 1 (FLT1); vascular endothelial growth factor (VEGF))

Relevant differences between ALDH⁺ and ALDH⁻ populations were noted for genes associated with angiogenesis faculty of WJ-MSCs. All the envisaged targets were less expressed in ALDH⁻ cells with mean expression of 1211 ± 41.5, 112 ± 8.1, $36{,}732 \pm 918$, $11{,}045 \pm 98.8$ compared with 3919 ± 250.4, 439.5 ± 16.5, $61{,}899 \pm 1520$, $15{,}593 \pm 398.5$ in ALDH⁺ cells for ANG1, ANG2, FLT1, VEGF respectively ($p = 0.0022$, $p = 0.0006$, $p = 0.0014$, $p = 0.0002$) (Fig. 6).

Figure 7: Hematopoietic support (matrix metalloproteinase 2 (MMP2); stromal derived factor 1 (SDF1); kit ligand (SCF); interleukin-6 (IL-6); IL-8)

Based on their ALDH activity, ALDH populations of WJ-MSCs presented distinct expressions of genes for hematopoiesis. ALDH$^+$ cells presented higher expression of IL6 and IL8 mRNA with respectively $450,699 \pm 5136$ and $75,812 \pm 6415$ compared to $247,255 \pm 5268$, $44,240 \pm 2001$ in ALDH$^-$ cells ($p < 0.0001$, $p = 0.0065$). MMP2, SDF1, and SCF presented the same profile ($261,792 \pm 9868$, 528.1 ± 12.2, $16,442 \pm 857.9$ in ALDH$^-$ cells versus $319,505 \pm 7234$, 660.8 ± 12, $25,809 \pm 977.1$ in ALDH$^+$ cells) (Fig. 7).

Figure 8: Immunomodulation (Galectin1 (GAL1); hepatocyte growth factor (HGF); leukemia inhibitory factor (LIF); cytochrome c oxidase subunit 1 (COX1), COX2)

Differences within WJ-MSCs immunoregulatory genes are dependent on ALDH activity. All the studied genes were most expressed in ALDH$^+$ cells compared to ALDH$^-$ cells. GAL1 was the most expressed in WJ-MSC ALDH$^+$ cells with $831,814 \pm 42,045$ compared to ALDH$^-$ $1,294,000 \pm 38,276$ ($p = 0.0034$). COX1 and COX2 were also significantly upregulated in ALDH$^+$ cells (354.4 ± 35, $147,693 \pm 2878$ versus 854.7 ± 13.65, $218,235 \pm 10,349$,
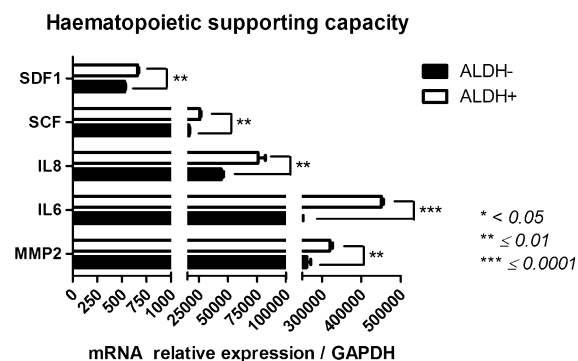
Haematopoietic supporting capacity

Fig. 7 Hematopoietic supporting capacity of ALDH$^+$ and ALDH$^-$ WJMSCs. After sorting ALDH$^+$ and ALDH$^-$ WJ-MSCs by FACS technique, we analyzed by qPCR their expression of *MMP2*, *SDF1*, *SCF*, *IL-6* and *IL-8* as illustration of their hematopoietic supporting capacity. Data are presented as mean $\pm$ SEM of mRNA gene expression relative to *GAPDH* expression
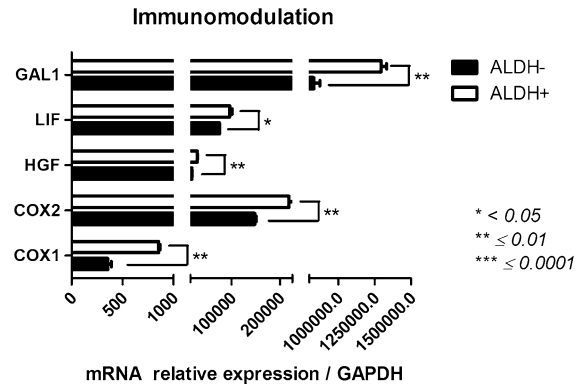
Immunomodulation

Fig. 8 Immunomodulatory ability of ALDH$^+$ and ALDH$^-$ WJ-MSCs. After sorting ALDH$^+$ and ALDH$^-$ WJ-MSCs by FACS technique, we examined by qPCR their expression of *GAL1*, *HGF*, *LIF*, *COX1* and *COX2* as demonstration of their immunomodulatory ability. Data are presented as mean $\pm$ SEM of mRNA gene expression relative to *GAPDH* expression

respectively). LIF and HGF presented the same profile with less expression in ALDH$^-$ population ($p = 0.0277$, $p = 0.0025$) (Fig. 8).

Trilineage potential (Fig. 9)

The gene expression illustrating the differentiation into either adipogenic, osteogenic or chondrogenic lineages was contrasting according to ALDH activity of both WJ-MSCs populations.

Adipogenesis potential (CCAAT/enhancer binding protein alpha (C/EBP-α), C/EBP-δ; Peroxisome proliferator activated receptor gamma (PPAR-γ); Kruppel like factor (KLF2); KLF5; Adiponectin (ADIPOQ)) The selection of the ALDH$^+$ and ALDH$^-$ populations made possible to isolate cell populations displaying different levels of *C/EBP-α* with a greater gene expression for the ALDH$^+$ subset. The levels of *C/EBP-α* was 551.2 ± 13 for ALDH$^+$ cells versus 305 ± 22.3 for ALDH$^-$ subset ($p = 0.0017$). Concerning *C/EBP-δ* gene expression, the ALDH$^+$ population showed the higher rate (886.9 ± 42.6) compared to the ALDH$^-$ population (299.8 ± 5.8) ($p = 0.0008$). Moreover, the levels of *KLF2* and *KLF5* were also upregulated in ALDH$^+$ compared to ALDH$^-$ populations (2680 ± 167.3 vs. 3681 ± 91.7 for KLF2) (146.4 ± 8.5 vs. 237.8 ± 6.1 for KLF5) ($p = 0.0095/p = 0.0003$). However, *PPARγ* and ADIPOQ were not significantly different (Fig. 9a).
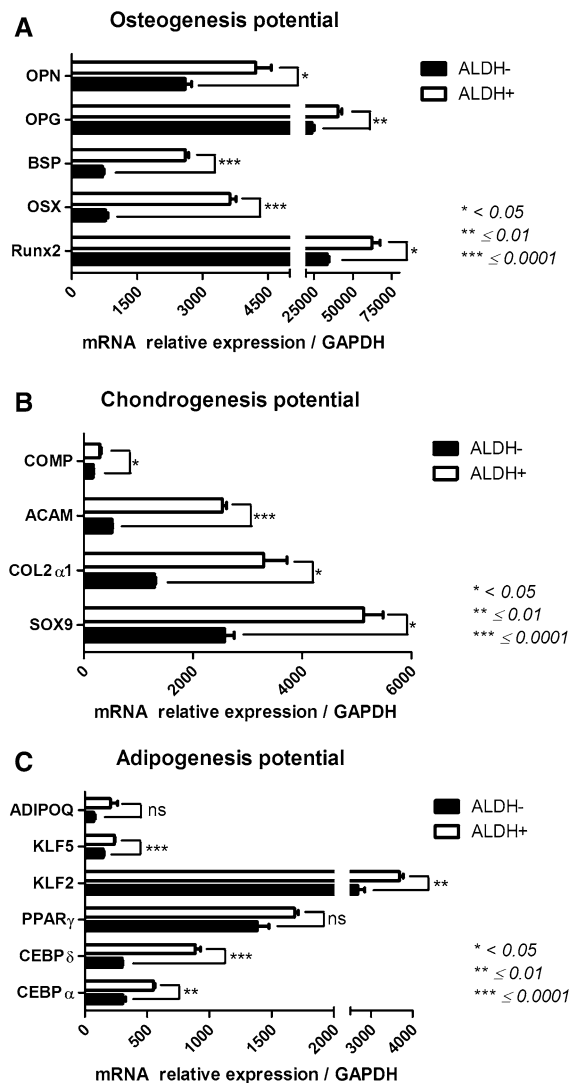
Fig. 9 Osteogenic competence of ALDH$^+$ and ALDH$^-$ WJ-MSCs. After sorting ALDH$^+$ and ALDH$^-$ WJ-MSCs by FACS technique, we explored by qPCR their expression of *Runx2*, *OSX*, *BSP*, *OPG* and *OPN* markers as manifestation of their aptitude to differentiate towards the osteogenic lineage (**a**). Concerning the chondrogenic competence of ALDH$^+$ and ALDH$^-$ WJ-MSCs, we checked the expression of *Sox9*, *Col2a1*, *ACAN* and *COMP* as reflection of their aptitude to differentiate towards the chondrogenic lineages (**b**). The adipogenic competence of ALDH$^+$ and ALDH$^-$ WJ-MSCs was analyzed and the expression of *C/EBP-α*, *C/EBP-δ*, *PPAR-γ*, *KLF2*, *KLF5* and *ADIPOQ* markers was evaluated as confirmation of their aptitude to differentiate towards the adipogenic lineages (**c**). Data are presented as mean ± SEM of mRNA gene expression relative to GAPDH expression

Osteogenesis potential (Runt related transcription factor 2 (Runx2); Osterix (OSX); Integrin binding sialoprotein (BSP); TNF receptor superfamily

member 11 (OPG); Secreted phosphoprotein type 1 (OPN)) The ALDH$^+$ population significantly showed the highest gene expression in ALDH$^+$ cells for *Runx2* (62,377 ± 5206), *BSP* (2606 ± 71.8), *OPG* (40,426 ± 2441) and *OPN* (4222 ± 359) genes compared to the ALDH$^-$ population (*Runx2*: 33,603 ± 1211, $p = 0.0102$; *BSP*: 706.5 ± 30.1, $p = 0.0003$; *OPG*: 23,858 ± 1419, $p = 0.0094$; *OPN*: 2600 ± 149.5, $p = 0.0104$) (Fig. 9b).

Chondrogenesis potential (Sry box 9 (Sox9); Collagen type II alpha 1 chain (Col2α1); Aggrecan (ACAN); Cartilage Oligomeric matrix protein (COMP)) ALDH$^-$ and ALDH$^+$ cells showed significant different expression of *Sox9* (2578 ± 178.2 and 5128 ± 354.9 respectively, $p = 0.0111$). Concerning *Col2α1* expression, the lowest expression was seen in the ALDH$^-$ population (1299 ± 21.9) in comparison to the ALDH$^+$ population (3295 ± 428.1, $p = 0.018$). For *ACAN* and *COMP* expressions, ALDH$^+$ population showed a higher expression than its negative counterpart ($p = 0.0001$ and $p = 0.0293$ respectively) (Fig. 9c).

Discussion

Their self-renewal, multilineage differentiation capacity and immunomodulatory abilities make mesenchymal stromal cells (MSCs) promising tools for different therapeutic applications. According to their origin, MSCs may share some characteristics (Hoogduijn et al. 2010) but may also present functional differences related to distinct gene and protein profiles (Busser et al. 2015; Fayyad-Kazan et al. 2017). The umbilical cord is becoming an important alternative source of MSCs (Davies et al. 2017). Wharton's jelly gelatin, firstly considered as a waste product, is currently fast obtained, non-invasive and abundant source of MSCs (Najar et al. 2016a; Beeravolu et al. 2017; Boey et al. 2017). Moreover this source already demonstrated benefic effects in cardio-regenerative medicine, in diabetes treatment or also in modulating Graft Versus Host Disease (Kamolz et al. 2014). Understanding the product profile of the intended therapy is crucial to guarantee a safe therapeutic use of MSCs (Sharpe et al. 2012; Atouf et al. 2013; De Kock et al. 2012). No marker is currently available for identifying MSCs from various tissue environments with distinct

competences to provide targeted efficient therapeutic (Najar et al. 2010a).

Aldehyde dehydrogenase (ALDH) activity was shown to be important in stem cell physiology (Ma and Allan 2011) and for tissue repair and regeneration (Kusuma et al. 2016; Kusuma et al. 2017; Sherman et al. 2017). Moreover, the activity and expression of ALDH seem to be a promising marker and potential therapeutic target for treating several malignancies (Rodriguez-Torres and Allan 2016). Thanks to their ability to potentiate cell therapy by being a marker of highly therapeutic MSCs, this family of cyto-protective enzymes is important to investigate. Complementary to our study, Vulcano and colleagues proved that WJ-MSCs induced lung cancer stem cell proliferation with higher ALDH$^+$ cells depending tumor type, these results suggest that the effect of WJ-MSC could be influenced by several factors (Vulcano et al. 2016). In this work, thanks to Fluorescence Activated Cell Sorting (FACS), we sorted two subsets of WJ-MSCs based on their ALDH activity (reported as ALDH$^+$ and ALDH$^-$). By harboring specific biological functionalities such as tissue repair activities and hematopoietic reconstitution, several cell progenitors with high ALDH activity have been shown to be therapeutically relevant (Balber 2011). The global gene expression profiles of a variety of non-hematopoietic progenitors are likely to have an important impact on their properties as well as on their cellular therapeutic application (De Kock et al. 2012). Both populations of WJ-MSCs demonstrated a distinct transcriptome profile (related to their ALDH activity) for genes associated with MSC major properties (stemness, cell-cycle, proliferation, phenotype, response to hypoxia, angiogenesis, multilineage capacities, immunomodulation and hematopoiesis support) (Kang et al. 2016; Stanko et al. 2014; Dolatabadi et al. 2017; Heo et al. 2016; Torensma et al. 2013). These differences between WJ-MSC populations may indicate biological features with high specific therapeutic relevance.

About the stemness (Rasini et al. 2013), Nanog, Rex1 and OCT-4 were more expressed in ALDH$^+$ WJ-MSCs compared to ALDH$^-$ counterpart. Our results were in accordance with previous studies reporting that these three genes where expressed by WJ-MSCs (Beeravolu et al. 2017; Van Pham et al. 2016).

Regarding cell cycle, we observed significant differences about the expression of CCNA, CCNB, CCNE, CDC25A, FosB, CDK1, CDK2, pRB, p16, p21 and p53. All the targets were increased in ALDH$^+$ cells compared to ALDH$^-$ cells. As the use of MSC in regenerative medicine may require large scale expansion, it's thus specially interesting to evaluate proliferation capacity of WJ-MSCs. Literature reported that the growth rate of WJ-MSCs was superior compared to the BM-MSCs due to higher proliferation with an upregulation of CCNA, CCNB, CDC25A and p53 (Batsali et al. 2017). The proliferation of WJ-MSCs has been influenced by obstetric factors (Wajid et al. 2015), themselves influenced by the In vitro passage of cells (Avercenc-Leger et al. 2017). These studies indicated that obstetric factors have to be considered to improve WJ-MSC proliferation. According our results WJ-MSC ALDH$^+$ cells seem to have more potential for proliferation, important aspect that should envisaged for therapeutic use. ALDH activity could influence the cell cycle and modulate properties of cellular population (Dolatabadi et al. 2017; Meng et al. 2014; Hegab et al. 2014). Mechanistically, ALDH throughout the catabolism of some endogenous substrates have the capacity to either stimulate or inhibit the expression of genes involved in cell cycle and therefore actively involved in controlling cell proliferation (Muzio et al. 2012).

The phenotype of a cellular product is an important issue for optimal cell-based therapy (New et al. 2015) and the phenotype of WJ-MSCs was often investigated (Van Pham et al. 2016; Walecka et al. 2017). Progenitors cells may display difference for CD54, 58, 106, 146 and CD200 depending of their origins (Najar et al. 2010a; Najar et al. 2012; Vishnubalaji et al. 2012; Huang et al. 2010). Here, we noticed a significant increased expression of CD54, 58, 106, 146 and CD200 in ALDH$^+$ WJ-MSCs. The expression of CD54, CD58 and CD106, main cell adhesion molecules, were shown to be tissue dependent (Najar et al. 2010a; Mandelli et al. 1999; Baer 2014). Interestingly, cells enriched in CD54 (Espagnolle et al. 2017) and CD106 (Yang et al. 2013; Du et al. 2016a) were shown to harbor great immunosuppressive capacities as well as potent pro-angiogenic abilities. Progenitor cells with enrichment on CD200 may also display distinguished immunomodulation (Najar et al. 2012), regenerative (Wang et al. 2014) and osteogenesis (Pontikoglou et al. 2016)

potentialities. In the other hand, striking stemness, multilineage and immunomodulatory capacities were reported for CD146 enriched cells (Wu et al. 2016).

The efficacy of MSC therapy is notably limited by poor survival of transplanted cells that face difficult microenvironment with low oxygen concentration. The ability of cells to response to hypoxia is another important point to evaluate. WJ-MSCs have been previously proved to don't be affected by ischemia and they thus be considered as a promising source for clinical applications (Himal et al. 2017). In this work, we observed an increased expression of HIF1α, HIF2α and GLUT1 in ALDH$^+$ population compared to ALDH$^-$ cells suggesting higher adaptation capacity to hypoxic environment. Hypoxia-inducible factors (HIFs) in a cell-type-specific manner are suggested to control different cellular functions such as angiogenesis, stemness and inflammation (Nauta et al. 2014; Bartels et al. 2013; D'Ippolito et al. 2006; Ohnishi et al. 2007). Thus, ALDH expression is likely involved in MSC resistance to oxidative stress (Kusuma et al. 2017).

MSCs have been widely proven effective for therapeutic angiogenesis in ischemia animal models as well as clinical vascular diseases. However, heterogeneous proangiogenic properties of MSCs derived from different tissue origins have been described and these differences are likely linked to the environment (Du et al. 2016b). In this study, we observed an increased expression for angiogenic genes ANG1, ANG2, FLT1 and VEGF in ALDH$^+$ WJ-MSCs. WJ-MSCs have been proved to possess pro angiogenic activity suggesting these cells as an attractive source for their use in tissue engineering (Arutyunyan et al. 2016; Edwards et al. 2014). Based on ALDH activity, as a part of their tissue repair and regenerative functions, subsets of MSCs according to their ALDH activity may promote angiogenesis by upregulating FLT1, CXCR4, and ANG2 (Pontikoglou et al. 2016; Nauta et al. 2014).

Homeostasis of the hematopoietic system is an important function enrolled by MSCs providing several factors [IL6, IL8, MMP2 and SCF (Fajardo-Orduna et al. 2015)] in the hematopoietic stem cell (HSC) niche. We highlighted that ALDH$^+$ subsets of WJ-MSCs present a potent hematopoiesis-supporting capacity by the significant up-regulation of SCF, SDF1, IL6, IL8 and MMP2 gene expression. By increasing the expression of MMP-2, IL-6 and IL-8

were involved in supporting the engraftment and migration of repopulating cells within the bone marrow (Briquet et al. 2010).

According to their immunological properties, WJ-MSCs are likely valuable candidates for allogeneic therapy (El Omar et al. 2014). Indeed, MSCs present immunomodulatory functions and therefore regulate the biology of several immune cells (Lee et al. 2012; Rizk et al. 2017; Abdi et al. 2008; Najar et al. 2010b; Purandare et al. 2014). ALDH$^+$ WJ-MSCs demonstrated increased expression of COX1-2, GAL1, LIF and HGF which indicate better immunomodulatory capacity and thus might be more attractive for immunotherapeutic interventions. These MSC derived factors are suggested to actively compete for the establishment of a tolerogenic state (Najar et al. 2016b). Accordingly, ALDH was shown to be critical for the induction of T-regs and thus promoting immunological tolerance (Steimle and Frick 2016).

We finally addressed the expression of the specific genes associated with their tri-lineage potential of MSCs (Rohban and Pieber 2017; Vella et al. 2011). Indeed, Wharton's jelly MSCs are endowed with high trilineage potential and thus are important for tissue repair and regeneration. In line with the results of (Zajdel et al. 2017), OSX, BSP, OPG, OPN and Runx2 were more expressed in ALDH$^+$ cells suggesting that this population clearly owned more capacities to differentiate into osteoclasts than ALDH$^-$ cells.

Concerning adipogenesis, Chen and colleagues showed that WJ-MSCs may differentiate into adipocytes and osteocytes (Chen et al. 2015). Counter that, Batsali and colleagues showed minor adipogenesis capacity in WJ-MSCs compared to BM-MSCs due to decreased expression of PPARg and CEBPa (Batsali et al. 2017). In our study, adipogenic markers CEBPα, CEBPδ, PPARγ, KLF2, KLF5 (Menssen et al. 2011; Lee and Ge 2014; Su et al. 2017) were most expressed in ALDH$^+$ WJ-MSCs.

We finally evaluated the potential differentiation capacity of WJ-MSCs into chondroblasts (Ronziere et al. 2010; Gonzalez-Fernandez et al. 2015). In this work, we demonstrated that ALDH$^+$ subsets presented more expression of chondrogenic genes (Sox9, ACAM, COMP, COL2α1). The chondrogenesis has been largely reported as WJ-MSC capacity (Baksh et al. 2007; Reppel et al. 2015). The work of Aleksander-Konert and colleagues notably proved the capacity of WJ-MSCs in chondrogenesis

(Aleksander-Konert et al. 2016) although literature proved that ALDH activity is not relevant for chondrogenesis capacity of MSCs (Estes et al. 2006).

Based on these results, we proved that ALDH$^+$ WJ-MSCs have more abilities to express pluripotent genes, to proliferate, to display potent immunomodulatory ability, to support hematopoiesis and angiogenesis, to response to hypoxia but also to differentiate into osteo-, chondro- and adipocyte-lineages compared to ALDH$^-$ population. This specific ALDH$^+$ subpopulation thus appears to be useful in numerous clinical applications. In addition to being a pivotal marker for stem/progenitor cell populations, the use of ALDH allow to identify a subset of WJ-MSCs with specific gene features pointing out that targeted therapeutic application based on these findings is feasible.

Authors' contributions Conceived and designed the experiments: MN, LL. Performed the experiments: MN, LL. Analyzed the data: EC, MN, LD. Contributed reagents/materials/analysis tools: LD, LVG. Wrote the paper: EC, MN, LL.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard This study was approved by the Bordet Institute Ethics Committee (Belgium) and conducted in accordance with the Declaration of Helsinki (1964). All donors and/or their parents gave written informed consent.

Availability of data and materials All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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