Data Article

Data supporting the activation of autophagy genes in the diabetic heart

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Abstract

This data article contains full list of autophagy related genes that are altered in diabetic heart. This article also shows data from in vitro cultured cardiomyocytes that are exposed the high glucose treatment to simulate hyperglycemic state in vitro. The interpretation of these data and further extensive insights into the regulation of SG biogenesis by AMPK can be found in “Type-2 diabetes increases autophagy in the human heart through promotion of Beclin-1 mediated pathway” (Munasinghe et al., in press) [1].

Specifications table

| Subject area      | Cardiovascular                        |
|-------------------|---------------------------------------|

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1. Value of the data

- First RT profiler array data for autophagy genes in the diabetic heart.
- In addition to beclin-1, RT profiler analysis of diabetic heart identified marked changes in several other genes. This could provide a benchmark for future research studies determining the pathophysiological role of other genes in autophagy.
- Isolated adult cardiomyocytes could be a valuable source to study the effect of diabetes in vitro.

2. Data, experimental design, materials and methods

2.1. Data

RT profiler array showed marked activation of several autophagy related genes with beclin-1 being markedly increased compared to other genes (Table 1). Importantly, exposure of adult cardiomyocytes to high glucose markedly increased the level of beclin-1 within 24 h with a peak increase at 48 h (Fig. 1A). Importantly, the caspase activation which indicates cell death followed the beclin-1 activation (Fig. 1B).

3. Experimental design, materials and methods

3.1. Animal model of type-2 diabetes

Male obese leptin–receptor mutant BKS.Cg-+Lepr<sup>db</sup>/+Lepr<sup>db</sup>/OlaHsd mice (Harlan, UK) were used as a model of insulin-resistant type-2 DM. Elevations of blood glucose begin at four to six weeks in these mutant mice. Age matched lean mice (BKS.Cg-m+/+Lepr<sup>db</sup>/OlaHsd) were used as control. These mice best represent the human model of type-2 diabetes [2,3].

3.2. RNA extraction and RT-profiler array for autophagy genes

Total RNA was extracted from the left ventricle of 8-weeks old diabetic and non-diabetic lean mice using Trizol, according to the manufacturer’s instructions (Invitrogen, UK). After confirming the purity and integrity of the total RNA, cDNA was prepared from 1 μg of total RNA by Transcription Kit (Qiagen, UK). The activation of apoptotic genes was then evaluated using murine RT-profiler PCR autophagy array (Qiagen, UK) using a light cycler (Roche 480, UK). Data were analyzed using the software...
package from Qiagen and expressed as fold-changes to control. Fold change of \( \geq 2 \) was considered significant [1,4,5].

3.3. Isolation and culture of adult cardiomyocytes

3.3.1. Isolation and culture of rat adult cardiomyocytes

The male Wistar rats were killed by cervical dislocation, the heart dissected and rinsed in cold solution A containing (in mM): 137 NaCl, 5 KCl, 1.2 MgSO\(_4\), 1.2 NaH\(_2\)PO\(_4\), 20 N-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES), 16 glucose, 5 Na pyruvate and 1.8 MgCl\(_2\) (pH 7.25 with NaOH) + 0.75 mM CaCl\(_2\). The heart was cannulated via the aorta and perfused for 4 min with solution A + 0.75 mM CaCl\(_2\) (all perfusing solutions were oxygenated and maintained at 37 °C). This was followed by a 4-min perfusion with solution A + 0.09 mM ethylene glycol-bis (β-aminoethyl ether) N,N,N,N'-tetraacetic acid (EGTA). Next the heart was digested with 50 ml of enzyme solution.

| Symbol | Fold Change | T-TEST | Fold Up- or Down-Regulation |
|--------|-------------|--------|-----------------------------|
| Thymoma viral proto-oncogene 1 | 1.00 | 0.448699 | -1.00 |
| Autophagy/beclin 1 regulator 1 | 1.20 | 0.301758 | 1.20 |
| Amyloid beta (A4) precursor protein | 0.76 | 0.438358 | -1.31 |
| Arylsulfatase A | 0.82 | 0.406747 | -1.21 |
| Autophagy-related 10 (yeast) | 1.35 | 0.253255 | 1.35 |
| Autophagy-related 12 (yeast) | 1.26 | 0.089850 | 1.26 |
| Autophagy-related 16-like 1 (yeast) | 1.02 | 0.755537 | 1.02 |
| Autophagy related 16 like 2 (S. cerevisiae) | 2.07 | 0.086888 | 2.07 |
| Autophagy-related 3 (yeast) | 2.39 | 0.033833 | 2.39 |
| Autophagy-related 4A (yeast) | 1.37 | 0.037771 | 1.37 |
| Autophagy-related 4B (yeast) | 1.32 | 0.223180 | 1.32 |
| Autophagy-related 4C (yeast) | 0.73 | 0.109416 | -1.37 |
| Autophagy-related 4D (yeast) | 0.92 | 0.453490 | -1.08 |
| Autophagy-related 5 (yeast) | 0.72 | 0.066282 | -1.39 |
| Autophagy-related 7 (yeast) | 0.84 | 0.562914 | -1.18 |
| Autophagy-related 9A (yeast) | 0.60 | 0.050481 | -1.66 |
| ATG9 autophagy related 9 homolog B (S. cerevisiae) | 0.80 | 0.051693 | -1.25 |
| BCL2-associated agonist of cell death | 1.34 | 0.040512 | 1.34 |
| BCL2-antagonist/killer 1 | 1.40 | 0.069000 | 1.40 |
| Bcl2-associated X protein | 0.08 | 0.003357 | -7.01 |
| B-cell leukemia/lymphoma 2 | 0.87 | 0.235046 | -1.15 |
| Bcl2-like 1 | 0.13 | 0.004101 | -7.59 |
| Beclin 1, autophagy related | 12.76 | 0.000041 | 12.76 |
| BH3 interacting domain death agonist | 2.47 | 0.004950 | 2.47 |
| BCL2 adenovirus E1B interacting protein 3 | 0.99 | 0.961009 | -1.01 |
| Caspase 3 | 5.88 | 0.000694 | 5.88 |
Table 1 (continued)

|                      | Value 1 | Value 2  | Value 3  |
|----------------------|---------|----------|----------|
| Caspase 8            | 0.89    | 0.859661 | -1.13    |
| Cyclin-dependent kinase inhibitor 1B | 0.71    | 0.241142 | -1.41    |
| Cyclin-dependent kinase inhibitor 2A | 0.84    | 0.129609 | -1.19    |
| Ceroid lipofuscinosis, neuronal 3, juvenile (Batten, Spielmeyer-Vogt disease) | 1.12    | 0.276092 | 1.12     |
| Cathespin B          | 0.81    | 0.490487 | -1.23    |
| Cathespin S          | 0.45    | 0.010346 | -2.20    |
| Chemokine (C-X-C motif) receptor 4 | 0.77    | 0.172117 | -1.30    |
| Death associated protein kinase 1 | 0.72    | 0.256786 | -1.40    |
| DNA-damage regulated autophagy modulator 1 | 0.88    | 0.662349 | -1.14    |
| Eukaryotic translation initiation factor 2 alpha kinase 3 | 0.82    | 0.131800 | -1.22    |
| Eukaryotic translation initiation factor 4, gamma 1 | 0.91    | 0.568731 | -1.10    |
| Estrogen receptor 1 (alpha) | 0.48    | 0.001831 | -2.08    |
| Fas (TNFRSF6)-associated via death domain | 0.85    | 0.301059 | -1.17    |
| Fas (TNF receptor superfamily member 6) | 1.14    | 0.519137 | 1.14     |
| Glucosidase, alpha, acid | 0.63    | 0.940482 | -1.58    |
| Gamma-aminobutyric acid receptor associated protein | 0.80    | 0.154834 | -1.25    |
| Gamma-aminobutyric acid (GABA) A receptor-associated protein-like 1 | 0.80    | 0.441529 | -1.26    |
| Gamma-aminobutyric acid (GABA) A receptor-associated protein-like 2 | 0.97    | 0.935761 | -1.03    |
| Histone deacetylase 1 | 0.62    | 0.217676 | -1.61    |
| HGF-regulated tyrosine kinase substrate | 0.57    | 0.073616 | -1.77    |
| Heat shock protein 90, alpha (cytosolic), class A member 1 | 0.73    | 0.010114 | -1.36    |
| Heat shock protein 8 | 0.81    | 0.000791 | -1.23    |
| Huntingtin | 0.62    | 0.398375 | -1.62    |
| Interferon alpha 2 | 0.58    | 0.125942 | -1.74    |
| Interferon alpha 4 | 0.31    | 0.010052 | -3.22    |
| Interferon gamma | 0.23    | 0.006485 | -4.26    |
| Insulin-like growth factor 1 | 0.59    | 0.000992 | -1.69    |
| Insulin II | 0.92    | 0.614254 | -1.09    |
| Immunity-related GTPase family M member 1 | 0.87    | 0.666668 | -1.15    |
| Microtubule-associated protein 1 light chain 3 alpha | 1.02    | 0.936707 | 1.02     |
| Microtubule-associated protein 1 light chain 3 beta (LC3B) | 6.98    | 0.000054 | 6.98     |
| Mitogen-activated protein kinase 14 | 0.74    | 0.007205 | -1.34    |
| Mitogen-activated protein kinase 8 | 0.71    | 0.000059 | -1.41    |
| Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105 | 0.71    | 0.012949 | -1.41    |
containing: solution A + 0.09 mM EGTA, 50 mg collagenase (Worthington Biochemical Corporation, Lakewood, New Jersey, USA. Type I), 5 mg protease (Sigma, Poole, Dorset, UK. Type IV), with (glutamate loaded) or without (control) 6.4 mM potassium L-glutamate until the tissue felt soft. There was a final 4-min perfusion with solution A + 0.15 mM CaCl₂ before the ventricles were cut down and sliced. The sliced ventricles were suspended in approximately 20–25 ml solution A + 0.15 mM CaCl₂ and shaken for 6 min at 37 °C. After filtration, cells were allowed to sediment, the supernatant was discarded, and the remaining cell layer suspended in solution A + 1 mM CaCl₂. This technique typically produced a yield of over 90% rod-shaped cells with the ability to exclude Trypan Blue [6]. The resulting cells were then washed separately with medium 199 (Invitrogen) supplemented with 0.2% BSA, 10% FBS, 5 mM creatine, 5 mM taurine, 2 mM carnitine, 10 μM cytosine-D-arabinofuranoside (all from Sigma chemicals), ITS and antibiotics (both from Invitrogen). After the final wash cells were resuspended in the same medium and plated on laminin coated culture dish according to the experiments [2].

Table 1 (continued)

| Gene Description                                      | Value 1 | Value 2 | Change |
|--------------------------------------------------------|---------|---------|--------|
| Phosphoinositide-3-kinase, class 3                     | 0.92    | 0.107642| -1.09  |
| Phosphoinositide-3-kinase, catalytic, gamma polypeptide| 0.64    | 0.182890| -1.57  |
| Phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 4, p150 | 0.71    | 0.009260| -1.40  |
| Protein kinase, AMP-activated, alpha 1 catalytic subunit | 2.39    | 0.002730| 2.39   |
| Protein kinase, AMP-activated, alpha 2 catalytic subunit | 1.20    | 0.415548| 1.20   |
| Phosphatase and tensin homolog                          | 1.09    | 0.608913| 1.09   |
| RAB24, member RAS oncogene family                      | 1.31    | 0.016826| 1.31   |
| Retinoblastoma 1                                        | 0.63    | 0.726162| -1.59  |
| Regulator of G-protein signaling 19                    | 0.84    | 0.239544| -1.18  |
| Ribosomal protein 56 kinase, polypeptide 1             | 0.65    | 0.036846| -1.53  |
| Synuclein, alpha                                        | 0.50    | 0.046069| -2.00  |
| Sequestosome 1                                          | 0.50    | 0.006853| -1.99  |
| Transforming growth factor, beta 1                     | 1.45    | 0.065043| 1.45   |
| Transglutaminase 2, C polypeptide                      | 0.80    | 0.708274| -1.24  |
| Family with sequence similarity 176, member A          | 0.86    | 0.255456| -1.16  |
| Transmembrane protein 74                               | 0.55    | 0.302192| -1.81  |
| VDNA-damage regulated autophagy modulator 2            | 0.78    | 0.159195| -1.28  |
| Tumor necrosis factor                                  | 0.94    | 0.709843| -1.06  |
| Tumor necrosis factor (ligand) superfamily, member 10 | 0.68    | 0.075484| -1.48  |
| Transformation related protein 53                     | 0.65    | 0.066200| -1.54  |
| Transformation related protein 73                      | 0.81    | 0.016038| -1.23  |
| Unc-51 like kinase 1 (C. elegans)                     | 0.75    | 0.016955| -1.33  |
| Unc-51 like kinase 2 (C. elegans)                     | 1.01    | 0.108460| 1.01   |
| UV radiation resistance associated gene                | 0.59    | 0.035331| -1.70  |
| Glucuronidase, beta                                    | 0.74    | 0.022172| -1.36  |
| Hypoxanthine guanine phosphoribosyl transferase        | 0.68    | 0.200702| -1.47  |
| Heat shock protein 90 alpha (cytosolic, class B member 1 | 0.42    | 0.013538| -2.36  |
| Glyceraldehyde-3-phosphate dehydrogenase               | 0.84    | 0.351495| -1.19  |
| Actin, beta                                            | 1.01    | 0.835590| 1.01   |

Highlighted in green are the significantly modulated autophagy related genes and highlighted in yellow are the significantly modulated cell survival related genes. Genes which are statistically significant (irrespective of the fold changes) are highlighted in red.
Effect of high glucose on beclin-1 expression

After isolation, 1 × 10^6 cardiomyocytes were seeded on a laminin-coated T25 flask and allowed to settle for 4 h. After 4 h, cardiomyocytes were exposed to high glucose (HG, 30 mM) or Mannitol (NG, 30 mM for osmotic control) to simulate a diabetic condition in vitro for 48 h. After 48 h, the effect of HG treatment on beclin-1 was measured by western blotting [7] and cell survival by caspase-3/7 activity as described earlier [2,8].

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2015.09.003.

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