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Insulin resistance in obese adolescents affects the expression of genes associated with immune response

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Objective. The development of obesity and its metabolic complications is associated with dysregulation of various intrinsic mechanisms, which control basic metabolic processes through changes in the expression of numerous regulatory genes.

Methods. The expression level of *HLA-DRA*, *HLA-DRB1*, *HLA-G*, *HLA-F*, and *NFX1* genes as well as miR-190b was measured in the blood of obese adolescents without signs of resistance to insulin and with insulin resistance in comparison with the group of relative healthy control individuals without signs of obesity.

Results. It was shown that obesity without signs of insulin resistance is associated with up-regulation of the expression level of *HLA-DRA* and *HLA-DRB1* genes, but with down-regulation of *HLA-G* gene expression in the blood as compared to control group of relative healthy adolescents. At the same time, no significant changes were observed in the expression level of *HLA-F* and *NFX1* genes in the blood of this group of obese adolescents. Development of insulin resistance in obese individuals leads to significant down-regulation of *HLA-DRA*, *HLA-DRB1*, *HLA-G*, and *HLA-F* gene expressions as well as to up-regulation of *NFX1* gene as well as microRNA miR-190b in the blood as compared to obese patients without signs of insulin resistance.

Conclusions. Results of this study provide evidence that obesity affects the expression of the subset of genes related to immune response in the blood and that development of insulin resistance in obese adolescents is associated with strong down-regulation of the expressions of *HLA-DRA*, *HLA-DRB1*, *HLA-F*, and *HLA-G* genes, which may be contribute to the development of obesity complications. It is possible that transcription factor *NFX1* and miR-190b participate in down-regulation of *HLA-DRA* gene expression in the blood of obese adolescents with insulin resistance.

Key words: mRNA expression, HLA-DRA, HLA-DRB1, HLA-F, HLA-G, NFX1, miR-190b, blood, obesity, insulin resistance

Obesity and its multiple complications including metabolic syndrome, insulin resistance, and type 2 diabetes represent the most profound public health problems, are associated with dysregulation of numerous regulatory mechanisms, which control the key metabolic pathways (Ruderman et al. 2013; Bass and Eneli 2015; Tam et al. 2017; Mao and Zhang 2018). The development of obesity and its numerous complications is a result from interactions between genes and environmental factors and is associated with endoplasmic reticulum stress and genome reprogramming in variable organs and tissues, but preferentially in adipose tissue and blood, which reflects numerous changes in different organs and tissues (Ozcan et al. 2004; Ando et al. 2011; Shimba et al. 2011; Wang and Kaufman 2016; Yamaoka et

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al. 2012; Han and Kaufman 2014; Lee and Ozcan 2014; Cajander et al. 2016). Special interest deserves the key factors, which control immune response as well as many other processes including cell proliferation (Schaiff et al. 1992; Nair et al. 2005; Kochan et al. 2013; Carosella et al. 2015; Wang et al. 2015, 2017; Boegel et al. 2018; Farjadian et al. 2018; Wu et al. 2017, 2018).

For this investigation following genes were selected: *HLA-DRA* (major histocompatibility complex, class II, DR alpha), *HLA-DRB1* (major histocompatibility complex, class II, DR alpha), *HLA-G* (major histocompatibility complex, class I, G), *HLA-F* (major histocompatibility complex, class I, F), and *NFX1* (nuclear transcription factor, X-box binding 1) as well as micro RNA miR-190b. The *HLA-DRA* and *HLA-DRB1* genes encode the alpha and beta1 subunits of HLA-DR receptor, which create a heterodimer. Both subunits anchored in the membrane and their co-regulated expression is mediated by the MHCII RNA operon and is controlled by FOXP1 as well as NFX1 (Song et al. 1994; Pisapia et al. 2013; Brown et al. 2016). Its primary role in immune response is presenting peptides derived from extracellular proteins. Furthermore, a reduction in *HLA-DR* gene expression, which is observed in cancer and in sepsis, correlates with an impaired TNFα response (Cajander et al. 2013; Leite et al. 2014; Winkler et al. 2017). The HLA-DRA mediates effects of insulin, insulin-like growth factor 1, and other cytokines by acting as a molecular adaptor between diverse receptor tyrosine kinases and downstream effectors through phosphorylation by the insulin receptor tyrosine kinase upon receptor stimulation as well as other kinases (Wu et al. 2017). It is interesting to note that in the proximal promoter of major histocompatibility complex (MHC) class II genes there is regulatory element, which sequence-specifically interacts with the conserved X1 box found in DNA binding protein NFX1, a cysteine-rich polypeptide, and regulates the expression of MHC class II molecules. There is data that overexpression of NFX1, which is a transcriptional repressor of MHC class II molecules, strongly and specifically represses the transcription of the *HLA-DRA* gene in MHC class II positive cell lines (Song et al. 1994). Furthermore, there is data that this transcription factor also participates in mammalian mRNA export as well as in TERT mRNA expression binds to the X-box motif of this gene promoter (Li and Noegel 2015; Yamashita et al. 2016).

The non-classical HLA-class I molecule G (human leukocyte antigen HLA-G) is a heterodimer consisting of a heavy chain and a light chain, which is involved in the presentation of foreign antigens to the immune system as well as in the susceptibility to cervical squamous cell carcinoma and other tumors (Yang et al. 2014; Zeestraten et al. 2014; Walentowicz-Sadlecka et al. 2018). Moreover, HLA-G when expressed by a tumor protects it from antitumor immunity (Carosella et al. 2015). It is interesting to note that alternative splicing of HLA-G transcripts yields proteins with primary structures resembling both class I and class II antigens (Ishitani and Geraghty 1992). There is data that HLA-G expression is associated with poor survival in stage III gastric cancer patients and represents a possible immunoescape mechanism of cancer cells (Murdaca et al. 2018). At the same time, there are two opposite sides of HLA-G function: beneficial to be promoted or deleterious to be blocked (Amiot et al. 2015; Carosella et al. 2015). Furthermore, the dual role of HLA-G in solid and liquid tumors was shown: HLA-G inhibits the proliferation of malignant B cells due to the interaction between HLA-G and its receptor ILT2, which mediates negative signaling on B cell proliferation (Rouas-Freiss et al. 2014). Recently, it was shown that there is a strong association between HLA-G 14 bp ins/ins genotype and insulin resistance (evaluated by homeostasis model assessment [HOMA]) in obese children and adolescents (Marzuillo et al. 2018).

Human leukocyte antigen HLA-F is a non-classical HLA-class I molecule with immunosuppressive properties. The overexpression of HLA-F in islet cells is a hallmark in the immunopathogenesis of type 1 diabetes (Richardson et al. 2016). Islet hyperexpression of HLA class I molecules occurred in the insulin-containing islets of patients with recent-onset type 1 diabetes and was also detectable in many patients with disease duration of up to 11 years, declining thereafter. Moreover, expression of HLA-F is correlated with tumor cell invasion and metastasis (Xu et al. 2013; Ishigami et al. 2015). HLA-F expression was found to be enhanced in gastric adenocarcinoma, breast cancer, esophageal squamous cell carcinoma, hepatocellular carcinoma, and neuroblastoma (Zhang et al. 2013; Morandi et al. 2013; Harada et al. 2015; Martinez-Canales et al. 2017; Wu et al. 2018).

The endoplasmic reticulum stress is also recognized as an important determinant of obesity, insulin resistance, and impaired glucose tolerance and contributes to the expression profile of many regulatory genes resulting in peripheral insulin resistance as well as other obesity complications (Ozcan et al. 2004; Wang and Kaufman 2012; Lee and Ozcan 2014; Hassler et al. 2015; Doultsinos et al. 2017; Wu et al. 2018), although detailed molecular mechanisms cannot be

ruled out. Activation of the endoplasmic reticulum stress sensors leads to transcriptional reprogramming of the cells, but inhibition of IRE1 mediated signaling pathway of endoplasmic reticulum stress affects the expression of numerous genes including insulin receptor and its substrates (IRS1 and IRS2), INSIG1 and INSIG2, insulin-like growth factors and IGF binding protein as well as proliferation related factors, estrogen and glucocorticoid related proteins (Auf et al. 2013; Chevet et al. 2015; Minchenko et al. 2013, 2015a, 2015b, 2016a, 2016b, 2017).

It is possible that clarification of real molecular mechanisms of metabolic disturbances in associated with obesity insulin resistance helps to better understanding why only a part of the obese individuals develops secondary metabolic disorders including resistance to insulin. However, a detailed molecular mechanism of the involvement of different genes of regulatory network in the development of insulin resistance and dysregulation of immune response in obese adolescents with insulin resistance remains unclear and deserves further investigation.

The main goal of this study was to clarify the role of the expression of a subset of genes, encoding for important immune response factors (*HLA-DRA*, *HLA-DRB1*, *HLA-F*, *HLA-G*, and *NFX1*) as well as microRNA miR-190b, in blood of obese adolescents with and without insulin resistance for evaluation of its probable significance in immune response in relation to obesity associated resistance to insulin.

Materials and methods

Patients' clinical characteristics. The 21 adolescents participate in this study. They were divided into three equal groups (7 subjects in each group): one group of relative healthy individuals without signs of obesity as control and two groups of obese patients: with and without insulin resistance. All participants gave written informed consent and the studies were approved by the local research ethics committees of Institute of Children and Adolescent Health Care of the National Academy of Medical Science of Ukraine.

Clinical characteristics of the study participants are shown in Table 1. The participants of control group were relative healthy individuals with mean age 14.2±0.584 years and mean body mass index (BMI) 18.8±0.17 kg/m^2. The obese participants without signs of insulin resistance had a mean age 13.8±0.265 years and a mean BMI 31.1±0.36 kg/m^2. The group of obese patients with insulin resistance was individuals with mean age 14.5±0.297 years and BMI 34.3±1.72 kg/m^2.

Thus, BMI, which is a main criterion of obesity, in both groups of obese patients was significantly higher (+66 in a group without signs of insulin resistance and +83% in a group with insulin resistance; $p<0.01$ in both cases) as compared to control individuals (Table 1). Furthermore, no significant changes were found in insulin resistance index evaluated by homeostasis model assessment (HOMA) in obese individuals as compared to relative healthy control group, but in obese patients with impaired insulin sensitivity the insulin resistance index was significantly increased as compared to control as well as to obese subjects with not changed insulin sensitivity (+258% and 225%, correspondingly; $p<0.001$ in both cases) (Table 1). The fasting insulin level did not significant changed in obese individuals with not changed insulin sensitivity, but strong increased in obese adolescents with insulin resistance as compared to relative healthy control individuals and to obese subjects without signs of insulin resistance (+232% and +197%, correspondingly; $p<0.001$). At the same time, the level of cortisol as well as fasting glucose did not change significantly in both groups of obese

Table 1
Clinical characteristics of the study participants

Variable	Control	Obesity	Obesity+IR
Age at visit (years)	14.2±0.584	13.8±0.265	14.5±0.297
Body mass index (BMI) (kg/m^2)	18.8±0.17	31.1±0.36*	34.3±1.72*
Insulin resistance index (HOMA)	2.38±0.128	2.63±0.219	8.51±0.99*^
Fasting insulin (μIU/ml)	12.8±0.69	14.3±0.95	42.5±4.69*^
Fasting glucose (mmol/l)	4.07±0.177	4.3±0.183	4.6±0.176
2-h oral glucose tolerance test (OGTT) glucose (mmol/l)	4.19±0.124	4.74±0.215	5.41±0.253*
Fasting cortisol (nmol/l)	602±30.11	553±22.87	512±22.75

Data are means ± SEM; n=7; *$p<0.05$ vs. control group; ^$p<0.05$ vs. obese group without insulin resistance.
Abbreviations: IR – insulin resistance.

adolescents regardless of the presence of insulin resistance as compared to control (Table 1). However, 2 h oral glucose tolerance test (OGTT) was significantly higher (+29%; $p<0.05$) only in the group of obese adolescents with insulin resistance as compared to control individuals (Table 1).

RNA isolation. Trisol reagent (Invitrogen, USA) was used for RNA extraction from the blood of healthy adolescents (without signs of obesity) as control and obese individuals with or without insulin resistance as described previously (Minchenko et al. 2004). The RNA pellets were washed with 75% ethanol and dissolved in ribonuclease-free water. For additional purification RNA samples were re-precipitated with 95% ethanol and re-dissolved in ribonuclease-free water. The concentration of RNA as well as its spectral characteristics was measured using NanoDrop Spectrophotometer ND1000 (PEQLAB, Biotechnologie GmbH).

Reverse transcription and quantitative real-time polymerase chain reaction analysis. The expression levels of genes related to the regulation of immune response (*HLA-DRA*, *HLA-DRB1*, *HLA-F*, *HLA-G*, and *NFX1*) as well as microRNA miR-190b as a potential regulator of HLA-DRA mRNA level were measured in blood by highly reproducible real-time quantitative polymerase chain reaction of cDNA (complementary DNA) (Cajander et al. 2016; Minchenko et al. 2018). Polymerase chain reaction was performed in triplicates. The "QuantStudio 5 Real-Time PCR System" (Applied Biosystems), Absolute QPCR SYBRGreen Mix (Thermo Scientific, UK) and pair of primers specific for each studied gene (Sigma-Aldrich, USA) was used for quantitative polymerase chain reaction (Table 2). For polyadenylation and reverse transcription of miRNAs we used NCode™ miRNA First-Strand cDNA Synthesis Kit MIRC-10 (Invitrogen, USA) with specific reverse primer included in this Kit. The level of microRNA miR-190b was measured by real-time qPCR using SYBR GreenER™ qPCR SuperMix Universal (Invitrogen) and specific for this microRNA forward primer (Table 2).

The expression of beta-actin mRNA was used as control of analyzed RNA quantity. The amplified DNA fragments were analyzed on a 2% agarose gel and that visualized by 5x Sight DNA Stain (EUROMEDEA). An analysis of quantitative PCR was performed using special computer program "Differential expression calculator".

Statistical analysis. Statistical analyses were performed according to Student's t-test using OriginPro 7.5 software (Bochkov et al. 2006). All values are expressed as mean±SEM from seven independent experiments; $p<0.05$ was considered as significant difference.

Results

We analyzed the expression of a subset of genes encoding HLA-DRA, HLA-DRB1, HLA-F, HLA-G, and NFX1 proteins, which play a central role in the immune system by presenting peptides derived from extracellular proteins and also associate with cell proliferation, in the blood of three groups of adolescents: relative healthy (control) participants, obese boys without signs of insulin resistance and obese individuals with insulin resistance using quantitative polymerase chain reaction. As shown in Figure 1, the expression level of *HLA-DRA* gene in the blood

Table 2
Characteristics of the primers used for quantitative real-time polymerase chain reaction

Gene symbol	Gene name	Primer's sequence	Nucleotide numbers in sequence	GenBank accession number
HLA-DRA	Major histocompatibility complex, class II, DR alpha	F: 5'- gaaagaaggagacggtctgg R: 5'- ctggtggggtgaacttgtct	294–313 630–511	NM_019111
HLA-DRB1	Major histocompatibility complex, class II, DR beta 1	F: 5'- aggcagcattgaagtcaggt R: 5'- ctcagcatcttgctctgtgc	350–369 604–585	NM_002124
HLA-F	Major histocompatibility complex, class I, F	F: 5'- atgcagaggagttcaggacc R: 5'- tctgtgtcctgggtctgttc	642–661 873–854	NM_018950
HLA-G	Major histocompatibility complex, class I, G	F: 5'- gcagagatacacgtgccatg R: 5'- agggcagctgtttcacattg	1012–1031 1240–1221	NM_002127
NFX1	nuclear transcription factor, X-box binding 1; transcriptional repressor	F: 5'- catcgatttccagaaccgcc R: 5'- cacgggaagttcttctgctg	2111–2130 2309–2290	NM_002504
ACTB	beta-actin	F: 5'- gacttcgagcaagagatgg R: 5'- gcactgtgttggcgtacag	747–766 980–961	NM_001101
miR-190b	microRNA miR-190b	F: 5'- tgatatgtttgatattgggttg	11–32	NM_030600

of obese adolescents with not changed sensitivity to insulin is increased (+21%; p<0.05) as compared to control group. At the same time, the expression level of this gene is significantly down-regulated in obese adolescents with insulin resistance as compared both to control group (–91%; p<0.001) and group of obese adolescents with not changed sensitivity to insulin (–93%; p<0.001) (Figure 1). Therefore, development of insulin resistance in obese adolescents strongly suppressed the expression of *HLA-DRA* gene in the blood. The *HLA-DRB1* gene encodes the beta1 subunits of HLA-DR receptor and its expression in obese adolescents was similar to *HLA-DRA* gene both in group without signs of insulin resistance and with insulin resistance (Figure 2). Thus, the expression level of *HLA-DRB1* gene is increased in the blood of obese individuals with not changed sensitivity to insulin (+23%; p<0.05) as compared to the group of healthy control adolescents. We have also shown that the development of insulin resistance in obese individuals leads to strong down-regulation of *HLA-DRB1* gene expression in the blood: –90% (p<0.001) as compared to control adolescents and –92% (p<0.001) as compared to the group of obese individuals without signs of insulin resistance (Figure 2). Therefore, development of insulin resistance in obese adolescents strongly suppressed the expression of *HLA-DRB1* gene like *HLA-DRA* gene, encoding protein-partner for heterodimeric HLA-DR receptor.

We also found that development of obesity with not changed sensitivity to insulin leads to down-regulation of the expression of *HLA-G* gene in the blood (–40%; p<0.01) as compared to control group of adolescents (Figure 3). More strong changes in this gene expression were observed in obese individuals with impaired sensitivity to insulin: –72% (p<0.001) adolescents and –53% (p<0.001) as compared to the group of obese individuals without sign of insulin resistance (Figure 3). Therefore, the expression of *HLA-G*, which is a non-classical HLA-class I molecule, is down-regulated in the blood of obese adolescents but development of insulin resistance in obese adolescents leads to more strongly suppression of this gene expression.

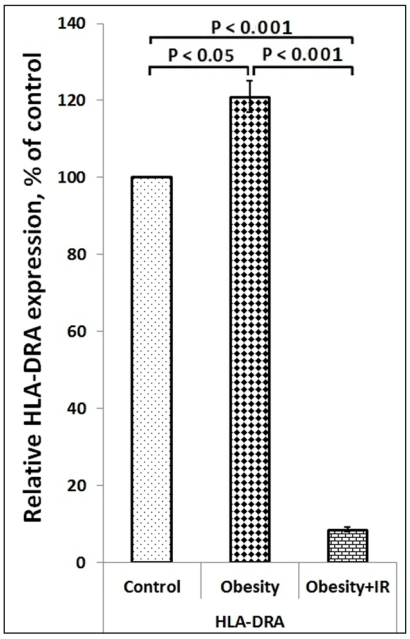

Figure 1. Expression of major histocompatibility complex, class II, DR alpha (HLA-DRA) mRNA in blood of obese individuals without insulin resistance (Obesity) and obese patients with resistance to insulin (Obesity + IR) as well as in normal adolescents (Control). The values of this mRNA expression were normalized to the expression of beta-actin mRNA, expressed as mean±SEM and represented as a percent of control (100%); n=7.

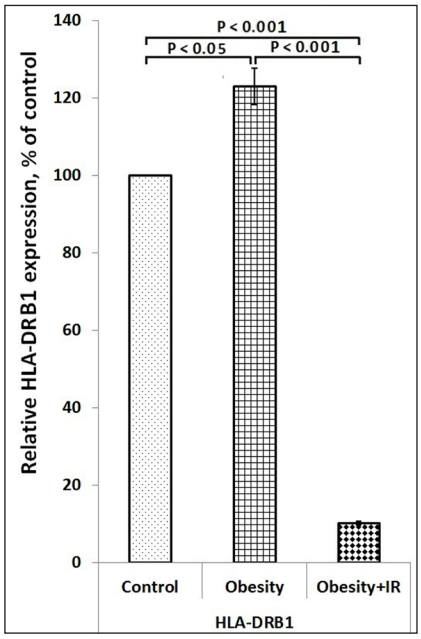

Figure 2. Expression of major histocompatibility complex, class II, DR beta 1 (HLA-DRB1) mRNA in blood of obese individuals without insulin resistance (Obesity) and obese patients with resistance to insulin (Obesity + IR) as well as in normal adolescents (Control). The values of this mRNA expression were normalized to the expression of beta-actin mRNA, expressed as mean±SEM and represented as a percent of control (100%); n=7.

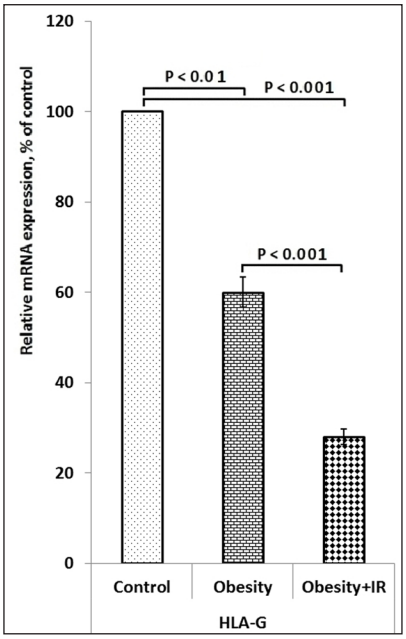

Figure 3. Expression of major histocompatibility complex, class I, G (HLA-G) mRNA in blood of obese individuals with normal insulin sensitivity (Obesity) and obese patients with insulin resistance (Obesity + IR) as well as in normal adolescents (Control). The values of this mRNA expression were normalized to the expression of beta-actin mRNA, expressed as mean±SEM and represented as a percent of control (100%); n=7.

As shown in Figure 4, the expression level of gene encoding *HLA-F*, which is a non-classical HLA-class I molecule with immunosuppressive properties, does not changed significantly in obese adolescents without sign of insulin resistance, but development of insulin resistance in obese adolescent leads to strong down-regulation of this gene expression in the blood: –89% ($p<0.001$) as compared to control individuals and –88% ($p<0.001$) as compared to the group of obese adolescents without sign of insulin resistance. Thus, the development of insulin resistance in obese adolescents strongly suppresses the expression level of *HLA-F* gene; meanwhile obesity does not affect its expression.

Next, we investigated the expression level of NFX1 mRNA in the blood of obese adolescents with not changed sensitivity to insulin and with insulin resistance, because NFX1 is a transcriptional repressor, which sequence-specifically interacts with the conserved X1 box regulatory element found in the proximal promoter of major histocompatibility complex (MHC) class II genes and regulates their expression (Song et al. 1994). As shown in Figure 5, the expression level of this gene does not change significantly in the group of obese individuals without signs of insulin resistance as compared to control group of adolescents. However, in obese individuals with impaired sensitivity to insulin we observed up-regulation of this gene expression (+17% ($p<0.05$) as compared to healthy control subjects and more significant changes in the expression of *NFX1* gene (+29%; $p<0.05$) as compared to the group of obese individuals without signs of insulin resistance (Figure 5). Therefore, the expression of *NFX1*, which is a transcriptional repressor, is up-regulated in the blood of obese adolescents with insulin resistance and possibly participates in strong suppression of *HLA-DRA* gene expression in the blood of obese adolescents with insulin resistance.

We have also studied the expression level of microRNA miR-190b because in 3'-region of HLA-DRA mRNA we identified specific binding site of this microRNA by bioinformatics analysis (http://www.targetscan.org/vert_50/). As shown in

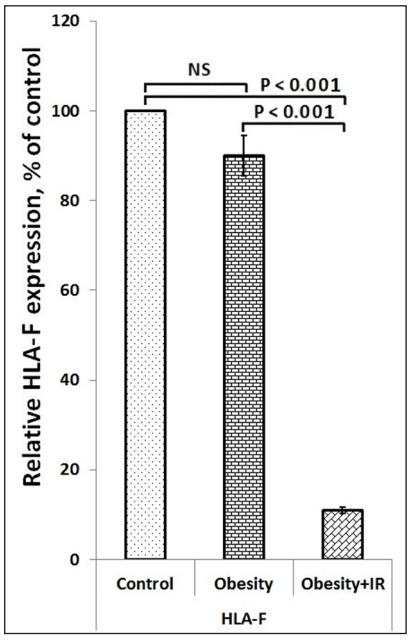

Figure 4. Expression of major histocompatibility complex, class I, F (HLA-F) mRNA in blood of obese individuals with normal insulin sensitivity (Obesity) and obese patients with insulin resistance (Obesity + IR) as well as in normal adolescents (Control). The values of this mRNA expression was normalized to the expression of beta-actin mRNA, expressed as mean±SEM and represented as a percent of control (100 %); n=7.

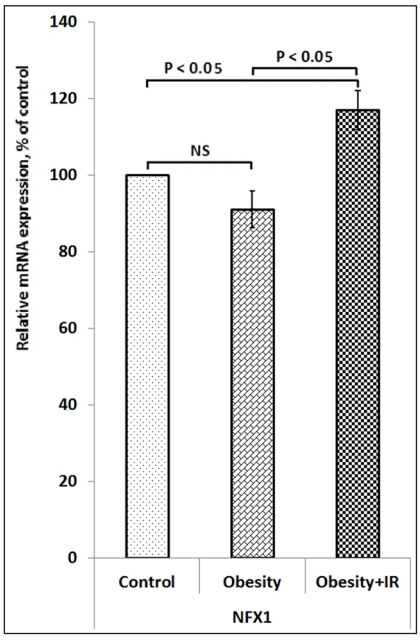

Figure 5. Expression of nuclear transcription factor, X-box binding 1 (NFX1) mRNA in blood of obese individuals without insulin resistance (Obesity) and obese patients with resistance to insulin (Obesity + IR) as well as in normal adolescents (Control). The values of this mRNA expression were normalized to the expression of beta-actin mRNA, expressed as mean±SEM and represented as a percent of control (100%); n=7.

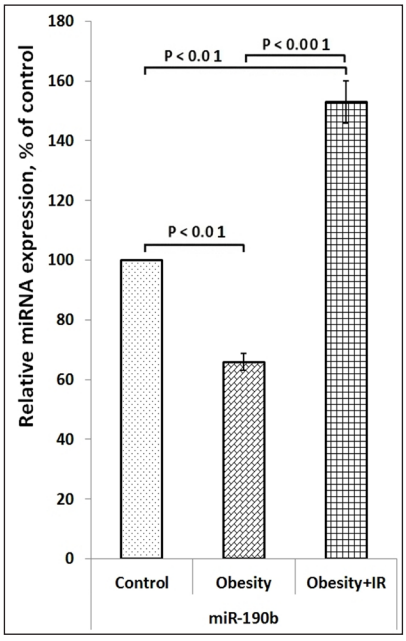

Figure 6. Expression of microRNA (miR-190b) in blood of obese individuals without insulin resistance (Obesity) and obese patients with resistance to insulin (Obesity + IR) as well as in normal adolescents (Control). The values of this miRNA expression were normalized to the expression of beta-actin mRNA, expressed as mean±SEM and represented as a percent of control (100%); n=7.

Figure 6, the level of miR-190b is decreased in the blood of obese adolescents with not changed sensitivity to insulin (–34%; p<0.01). At the same time, development of insulin resistance leads to reversible changes in this microRNA expression: +53% (p<0.01) as compared to control group of healthy individuals and +132% (p<0.001) as compared to the group of obese adolescents without signs of insulin resistance (Figure 6). Thus, the changes in miR-190b possibly contribute both in up-regulation of *HLA-DRA* gene expression in the blood of obese adolescents without signs of insulin resistance and in strong suppression of this gene expression in subjects with obesity complicated by insulin resistance.

Discussion

Results of this investigation clearly demonstrated that obesity affects the expression level of most studied immune response related genes in gene-specific manner in the blood and that these changes are insulin sensitive because development of insulin resistance in obese individuals significantly modifies these genes expression levels (Figure 7). Thus, the expression level of *HLA-DRA* and *HLA-DRB1* genes is increased in obese adolescents and these results agree well with data Nair et al. (2005) that obesity upregulates HLA-DRA expression. However, development of insulin resistance in obese individuals strongly suppressed the expression of both *HLA-DRA* and *HLA-DRB1* genes. These results indicate that these genes expression is very sensitive to the efficiency of insulin action. The changes in the expression of both *HLA-DRA* and *HLA-DRB1* genes are similar in obese adolescents without signs of insulin resistance as well as with insulin resistance. This similarity can be explained their co-regulated expression, which is mediated by the MHCII RNA operon (Song et al. 1994; Pisapia et al. 2013). It is possible, at least in part, that upregulation of the expression of *HLA-DRA* gene in the blood of obese adolescents is associated with down-regulation of miR-190b, which has specific binding sites on 3'-untranslated region of HLA-DRA mRNA. Furthermore, down-regulation of *HLA-DRA* gene expression in obese subjects with insulin resistance possibly associated, at least in part, with up-regulation of transcription repressor NFX1 as well as significant increase of microRNA miR-

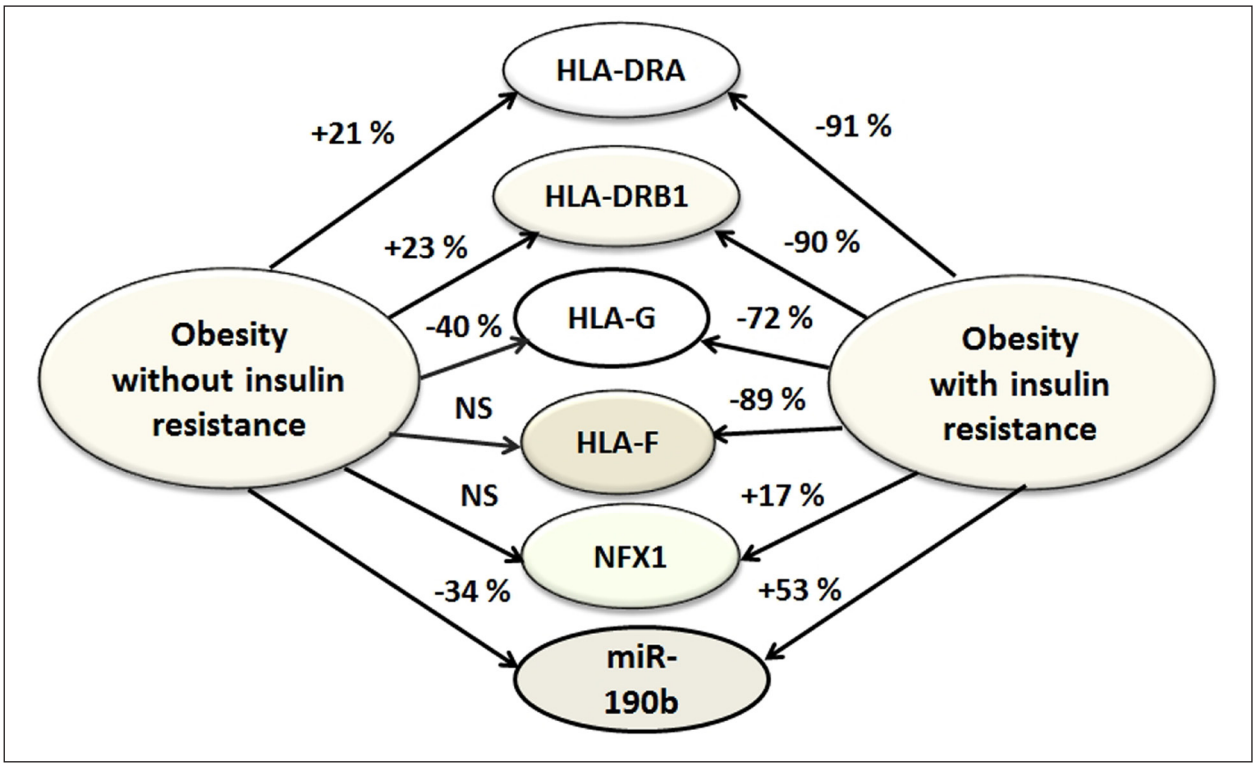

Figure 7. Schematic demonstration of *HLA-DRA*, *HLA-DRB1*, *HLA-G*, *HLA-F*, and *NFX1* genes as well as *miR-190b* expression profile in the blood of obese adolescences with not changed insulin sensitivity and with insulin resistance as compared to control group of healthy subjects.

190b. These results agree well with data Song et al. (1994) because down-regulation of *HLA-DRA* gene expression is associated with up-regulation of transcription repressor NFX1. It is interesting to note that the RTA (replication and transcription activator) encoded by Kaposi's sarcoma-associated herpesvirus can function as an E3 ligase to degrade HLA-DRA by direct binding to HLA-DRA and inducing its degradation of through the ubiquitin-proteasome degradation pathway (Sun et al. 2016).

At the same time, the expression level of non-classical HLA-class I molecule G (HLA-G) is down-regulated in childhood obesity and in obese adolescents with insulin resistance these changes were more significant. Recently, it was shown that there is a strong association between HLA-G 14 bp ins/ins genotype and insulin resistance in obese children and adolescents (Marzuillo et al. 2018), but at the same time, there were identified two opposite sides of HLA-G function: beneficial to be promoted or deleterious to be blocked (Amiot et al. 2015; Carosella et al. 2015). Thus, additional studies may be necessary to determine the functional significance of *HLA-G* gene expression in obese subjects both with and without insulin resistance.

Next, we have shown that the expression of other representative of non-classical HLA-class I molecules, HLA-F, is down-regulated in obese adolescents only after development of insulin resistance. It is possible that *HLA-F* gene expression is very sensitive to the effectiveness of insulin action. At the same time, we could not exclude other mechanisms of strong down-regulation of this gene expression in obesity associated insulin resistance. It is interesting to note that the overexpression of HLA-F in islet cells is a hallmark in the immunopathogenesis of type 1 diabetes (Richardson et al. 2016). It is possible that HLA-F has additional function because these are also findings that HLA-F expression is correlated with tumor cell invasion and metastasis (Xu et al. 2013; Ishigami et al. 2015; Martinez-Canales et al. 2017; Wu et al. 2018). Thus, the functional significance of insulin resistance associated changes in the expression of *HLA-F* gene, as a non-classical HLA-class I molecule with immunosuppressive properties, remains unclear and deserves further investigation.

The development of obesity as well as its metabolic complications is associated with dysregulation of numerous intrinsic mechanisms, which control most key metabolic processes, including cellular growth, glucose and lipid metabolism as well as insulin sensitivity and immune response (Ruderman et al. 2013; Lee and Ozcan 2014; Leite et al. 2014; Tam et al. 2017; Wu et al. 2017). It is possible that changes in classical HLA-class II molecules and non-classical HLA-class I molecules, which we observed in childhood obesity with and without insulin resistance, can contribute to these numerous intrinsic mechanisms of glucose and lipid metabolism dysregulation as well as cellular growth, because HLA-DR receptor and non-classical HLA-class I molecules play an important role in the regulation of both immune response and proliferative processes and participate in endoplasmic reticulum stress, an important factor of insulin resistance and obesity (Schaiff et al. 1992; Minchenko et al. 2005; Yuzefovych et al. 2013; Han and Kaufman 2014; Lee and Ozcan 2014; Carosella et al. 2015; Walentowicz-Sadlecka et al. 2018; Yang et al. 2018). Furthermore, there is data that both HLA-DR receptor and non-classical HLA-class I molecules play an important role in tumorigenesis (Kochan et al. 2013; Morandi et al. 2013; Leite et al. 2014; Zeestraten et al. 2014; Harada et al. 2015; Ishigami et al. 2015; Brown et al. 2016; Martinez-Canales et al. 2017; Winkler et al. 2017; Walentowicz-Sadlecka et al. 2018; Wu et al. 2018). It is interesting to note that the absent in melanoma 2 (AIM2), which is a member of the IFN-inducible nuclear proteins, associated with both infection defense and tumor pathology, mediates induction of several interferon-stimulated genes, including genes encoding the major histocompatibility complex class II antigens HLA-DRA and HLA-DRB in cancer cells (Kochan et al. 2013). In this study, we have shown that obesity down-regulates the expression of miR-190b but development of insulin resistance in obese adolescents leads to significant up-regulation of this microRNA level, which possibly contributes to strong down-regulation of HLA-DRA as well as in many other processes, because there is data that miR-190b is significantly up-regulated in breast tumors (Cizeron-Clairac et al. 2015). These data completely agree with our results, which demonstrated possible mechanism connecting obesity with tumorigenesis as well as with data Tsuchihara et al. (2008).

Taking into account the importance of the major histocompatibility complex class II and class I antigen functions, we suggest that down-regulation of the expression levels of *HLA-DRA*, *HLA-DRB1*, *HLA-G*, and *HLA-F* as well as up-regulation of miR-190b mediated by insulin resistance associated with obesity may contribute to the dysregulation of immune response as well as to the risk of tumorigenesis. Therefore, it is possible that most obesity and insulin related changes in the expression of the major histocompatibility complex class II and class

I antigen genes are mediated through endoplasmic reticulum stress, although detailed molecular mechanisms cannot be ruled out.

We hope that identification of real mechanisms of metabolic abnormalities in obesity as well as its complications at molecular and cellular levels helps to better understanding why obesity develops and why only a part of the obese individuals develops secondary metabolic disorders including insulin resistance and dysregulation of immune response. However, a detailed molecular mechanism of the involvement of different genes of regulatory network in the development of obesity and its complications are not clear yet and remains to be determined.

References

Amiot L, Vu N, Samson M. Biology of the immunomodulatory molecule HLA-G in human liver diseases. J Hepatol 62, 1430–1437, 2015.

Ando H, Kumazaki M, Motosugi Y, Ushijima K, Maekawa T, Ishikawa E, Fujimura A. Impairment of peripheral circadian clocks precedes metabolic abnormalities in ob/ob mice. Endocrinology 152, 1347–1354, 2011.

Auf G, Jabouille A, Delugin M, Guerit S, Pineau R, North S, Platonova N, Maitre M, Favereaux A, Vajkoczy P, Seno M, Bikfalvi A, Minchenko D, Minchenko O, Moenner M. High epiregulin expression in human U87 glioma cells relies on IRE1 alpha and promotes autocrine growth through EGF receptor. BMC Cancer 13, 597, 2013.

Bass R, Eneli I. Severe childhood obesity: an under-recognised and growing health problem. Postgrad Med J 91, 639–645, 2015.

Bochkov VN, Philippova M, Oskolkova O, Kadl A, Furnkranz A, Karabeg E, Breuss J, Minchenko OH, Mechtcheriakova D, Hohensinner P, Rychli K, Wojta J, Resink T, Binder BR, Leitinger N. Oxidized phospholipids stimulate angiogenesis via induction of VEGF, IL-8, COX-2 and ADAMTS-1 metalloprotease, implicating a novel role for lipid oxidation in progression and destabilization of atherosclerotic lesions. Circ Res 99, 900–908, 2006.

Boegel S, Lower M, Bukur T, Sorn P, Castle JC, Sahin U. HLA and proteasome expression body map. BMC Med Genomics 11, 36, 2018.

Brown PJ, Wong KK, Felce SL, Lyne L, Spearman H, Soilleux EJ, Pedersen LM, Moller MB, Green TM, Gascoyne DM, Banham AH. FOXP1 suppresses immune response signatures and MHC class II expression in activated B-cell-like diffuse large B-cell lymphomas. Leukemia 30, 605–616, 2016.

Cajander S, Backman A, Tina E, Stralin K, Soderquist B, Kallman J. Preliminary results in quantitation of HLA-DRA by real-time PCR: a promising approach to identify immunosuppression in sepsis. Crit Care 17, R223, 2013.

Cajander S, Tina E, Backman A, Magnuson A, Stralin K, Soderquist B, Kallman J. Quantitative real-time polymerase chain reaction measurement of HLA-DRA gene expression in whole blood is highly reproducible and shows changes that reflect dynamic shifts in monocyte surface HLA-DR expression during the course of sepsis. PLoS One 11, e0154690, 2016.

Carosella ED, Rouas-Freiss N, Tronik-Le Roux D, Moreau P, LeMaoult J. HLA-G: an immune checkpoint molecule. Adv Immunol 127, 33–144, 2015.

Chevet E, Hetz C, Samali A. Endoplasmic reticulum stress-activated cell reprogramming in oncogenesis. Cancer Discov 5, 586–597, 2015.

Cizeron-Clairac G, Lallemand F, Vacher S, Lidereau R, Bieche I, Callens C. MiR-190b, the highest up-regulated miRNA in ERalpha-positive compared to ERalpha-negative breast tumors, a new biomarker in breast cancers? BMC Cancer 15, 499, 2015.

Doultsinos D, Avril T, Lhomond S, Dejeans N, Guedat P, Chevet E. Control of the unfolded protein response in health and disease. SLAS Discov 22, 787–800, 2017.

Farjadian S, Tabebordbar M, Mokhtari M, Safaei A, Malekzadeh M, Ghaderi A. HLA-G expression in tumor tissues and soluble HLA-G plasma levels in patients with gastrointestinal cancer. Asian Pac J Cancer Prev 19, 2731–2735, 2018.

Han J, Kaufman RJ. Measurement of the unfolded protein response to investigate its role in adipogenesis and obesity. Methods Enzymol 538, 135–150, 2014.

Harada A, Ishigami S, Kijima Y, Nakajo A, Arigami T, Kurahara H, Kita Y, Yoshinaka H, Natsugoe S. Clinical implication of human leukocyte antigen (HLA)-F expression in breast cancer. Pathol Int 65, 569–574, 2015.

Hassler JR, Scheuner DL, Wang S, Han J, Kodali VK, Li P, Nguyen J, George JS, Davis C, Wu SP, Bai Y, Sartor M, Cavalcoli J, Malhi H, Baudouin G, Zhang Y, Yates Iii JR, Itkin-Ansari P, Volkmann N, Kaufman RJ. The IRE1α/XBP1s pathway is essential for the glucose response and protection of β cells. PLoS Biol 13, e1002277, 2015.

Ishigami S, Arigami T, Okumura H, Uchikado Y, Kita Y, Kurahara H, Maemura K, Kijima Y, Ishihara Y, Sasaki K, Uenosono Y, Natsugoe S. Human leukocyte antigen (HLA)-E and HLA-F expression in gastric cancer. Anticancer Res 35, 2279–2285, 2015.

Ishitani A, Geraghty DE. Alternative splicing of HLA-G transcripts yields proteins with primary structures resembling both class I and class II antigens. Proc Natl Acad Sci USA 89, 3947–3951, 1992.

Kochan G, Escors D, Breckpot K, Guerrero-Setas D. Role of non-classical MHC class I molecules in cancer immunosuppression. Oncoimmunology 2, e26491, 2013.

Lee J, Ozcan U. Unfolded protein response signaling and metabolic diseases. J Biol Chem 289, 1203–1211, 2014.

Leite FA, Lira RC, Fedatto PF, Antonini SR, Martinelli CE Jr, de Castro M, Neder L, Ramalho LN, Tucci S Jr, Mastelaro MJ, Seidinger AL, Cardinalli IA, Yunes JA, Brandalise SR, Tone LG, Scrideli CA. Low expression of HLA-DRA, HLA-DPA1, and HLA-DPB1 is associated with poor prognosis in pediatric adrenocortical tumors (ACT). Pediatr Blood Cancer 61, 1940–1948, 2014.

Li P, Noegel AA. Inner nuclear envelope protein SUN1 plays a prominent role in mammalian mRNA export. Nucleic Acids Res 43, 9874–9888, 2015.

Mao Z, Zhang W. Role of mTOR in glucose and lipid metabolism. Int J Mol Sci 19, pii: E2043, 2018.

Martinez-Canales S, Cifuentes F, Lopez De Rodas Gregorio M, Serrano-Oviedo L, Galan-Moya EM, Amir E, Pandiella A, Gyorffy B, Ocana A. Transcriptomic immunologic signature associated with favorable clinical outcome in basal-like breast tumors. PLoS One 12, e0175128, 2017.

Marzuillo P, Bellini G, Punzo F, Di Sessa A, Guarino S, Umano GR, Toraldo R, Miraglia Del Giudice E, Rossi F. Association between 14 bp insertion/deletion HLA-G functional polymorphism and insulin resistance in a cohort of Italian children with obesity. Pediatr Diabetes 19, 1357–1361, 2018.

Minchenko DO, Kharkova AP, Hubenia OV, Minchenko OH. Insulin receptor, IRS1, IRS2, INSIG1, INSIG2, RRAD, and BAIAP2 gene expressions in glioma U87 cells with ERN1 loss of function: effect of hypoxia and glutamine or glucose deprivation. Endocr Regul 47, 15–26, 2013.

Minchenko DO, Kharkova AP, Karbovskyi LL, Minchenko OH. Expression of insulin-like growth factor binding protein genes and its hypoxic regulation in U87 glioma cells depends on ERN1 mediated signaling pathway of endoplasmic reticulum stress. Endocr Regul 49, 73–83, 2015a.

Minchenko DO, Kharkova AP, Halkin OV, Karbovskyi LL, Minchenko OH. Effect of hypoxia on the expression of genes encoded insulin-like growth factors and some related proteins in U87 glioma cells without IRE1 function. Endocr Regul 50, 43–54, 2016a.

Minchenko DO, Riabovol OO, Tsymbal DO, Ratushna OO, Minchenko OH. Inhibition of IRE1 affects the expression of genes encoded glucocorticoid receptor and some related factors and their hypoxic regulation in U87 glioma cells. Endocr Regul 50, 127–136, 2016b.

Minchenko DO, Riabovol OO, Ratushna OO, Minchenko OH. Hypoxic regulation of the expression of genes encoded estrogen related proteins in U87 glioma cells: effect of IRE1 inhibition. Endocr Regul 51, 8–19, 2017.

Minchenko OH, Opentanova IL, Minchenko DO, Ogura T, Esumi H. Hypoxia induces transcription of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 gene via hypoxia-inducible factor-1alpha activation. FEBS Lett 576, 14–20, 2004.

Minchenko OH, Ochiai A, Opentanova IL, Ogura T, Minchenko DO, Caro J, Komisarenko SV, Esumi H. Overexpression of 6-phosphofructo-2-kinase/fructose-2,6- bisphosphatase-4 in the human breast and colon malignant tumors. Biochimie 87, 1005–1010, 2005.

Minchenko OH, Tsymbal DO, Minchenko DO, Moenner M, Kovalevska OV, Lypova NM. Inhibition of the endoribonuclease of ERN1 signaling enzyme affects the expression of proliferation-related genes in U87 glioma cells. Endoplasm Reticul Stress Dis 2, 18–29, 2015b.

Minchenko OH, Tsymbal DO Minchenko DO, Hnatiuk OS, Prylutskyy YI, Prylutska SV, Ritter U. Suppression of the expression of genes associated with immune response in normal human astrocytes upon treatment by single-walled carbon nanotubes. Toxicol In Vitro 52, 122–130, 2018.

Morandi F, Cangemi G, Barco S, Amoroso L, Giuliano M, Gigliotti AR, Pistoia V, Corrias MV. Plasma levels of soluble HLA-E and HLA-F at diagnosis may predict overall survival of neuroblastoma patients. Biomed Res Int 2013, 956878, 2013.

Murdaca G, Calamaro P, Lantieri F, Pigozzi S, Mastracci L, Grillo F, Magnani O, Ceppa P, Puppo F, Fiocca R. HLA-G expression in gastric carcinoma: clinicopathological correlations and prognostic impact. Virchows Arch 473, 425–433, 2018.

Nair S, Lee YH, Rousseau E, Cam M, Tataranni PA, Baier LJ, Bogardus C, Permana PA. Increased expression of inflammation-related genes in cultured preadipocytes/stromal vascular cells from obese compared with non-obese Pima Indians. Diabetologia 48, 1784–1788, 2005.

Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 306, 457–461, 2004.

Pisapia L, Cicatiello V, Barba P, Malanga D, Maffei A, Hamilton RS, Del Pozzo G. Co-regulated expression of alpha and beta mRNAs encoding HLA-DR surface heterodimers is mediated by the MHCII RNA operon. Nucleic Acids Res 41, 3772–3786, 2013.

Richardson SJ, Rodriguez-Calvo T, Gerling IC, Mathews CE, Kaddis JS, Russell MA, Zeissler M, Leete P, Krogvold L, Dahl-Jorgensen K, von Herrath M, Pugliese A, Atkinson MA, Morgan NG. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. Diabetologia 59, 2448–2458, 2016.

Rouas-Freiss N, Moreau P, LeMaoult J, Carosella ED. The dual role of HLA-G in cancer. J Immunol Res 2014, 359748, 2014.

Ruderman NB, Carling D, Prentki M, Cacicedo JM. AMPK, insulin resistance, and the metabolic syndrome. J Clin Invest 123, 2764–2772, 2013.

Schaiff WT, Hruska KA Jr, McCourt DW, Green M, Schwartz BD. HLA-DR associates with specific stress proteins and is retained in the endoplasmic reticulum in invariant chain negative cells. J Exp Med 176, 657–666, 1992.

Shimba S, Ogawa T, Hitosugi S, Ichihashi Y, Nakadaira Y, Kobayashi M, Tezuka M, Kosuge Y, Ishige K, Ito Y, Komiyama K, Okamatsu-Ogura Y, Kimura K, Saito M. Deficient of a clock gene, brain and muscle Arnt-like protein-1 (BMAL1), induces dyslipidemia and ectopic fat formation. PLoS One 6, e25231, 2011.

Song Z, Krishna S, Thanos D, Strominger JL, Ono SJ. A novel cysteine-rich sequence-specific DNA-binding protein interacts with the conserved X-box motif of the human major histocompatibility complex class II genes via a repeated Cys-His domain and functions as a transcriptional repressor. J Exp Med 180, 1763–1774, 1994.

Sun Z, Jha HC, Pei YG, Robertson ES. Major histocompatibility complex class II HLA-DRα is downregulated by Kaposi's sarcoma-associated herpesvirus-encoded lytic transactivator RTA and MARCH8. J Virol 90, 8047–8058, 2016.

Tam J, Szanda G, Drori A, Liu Z, Cinar R, Kashiwaya Y, Reitman ML, Kunos G. Peripheral cannabinoid-1 receptor blockade restores hypothalamic leptin signaling. Mol Metab 6, 1113–1125, 2017.

Tsuchihara K, Ogura T, Fujioka R, Fujii S, Kuga W, Saito M, Ochiya T, Ochiai A, Esumi H. Susceptibility of Snark-deficient mice to azoxymethaneinduced colorectal tumorigenesis and the formation of aberrant crypt foci. Cancer Sci 99, 677–682, 2008.

Walentowicz-Sadlecka M, Dziobek K, Grabiec M, Sadlecki P, Walentowicz P, Mak P, Szymankiewicz M, Kwinta P, Dutsch-Wicherek M. The analysis of human leukocyte antigen-G level in patients with endometrial cancer by Western blot technique. Am J Reprod Immunol 10, e13070, 2018.

Wang C, Cai L, Liu J, Wang G, Li H, Wang X, Xu W, Ren M, Feng L, Liu P, Zhang C. MicroRNA-30a-5p inhibits the growth of renal cell carcinoma by modulating GRP78 expression. Cell Physiol Biochem 43, 2405–2419, 2017.

Wang L, Wei B, Hu G, Wang L, Bi M, Sun Z, Jin Y. Screening of differentially expressed genes associated with human glioblastoma and functional analysis using a DNA microarray. Mol Med Rep 12, 1991–1996, 2015.

Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature 529, 326–335, 2016.

Wang S, Kaufman RJ. The impact of the unfolded protein response on human disease. J Cell Biol 197, 857–867, 2012.

Winkler MS, Rissiek A, Priefler M, Schwedhelm E, Robbe L, Bauer A, Zahrte C, Zoellner C, Kluge S, Nierhaus A. Human leucocyte antigen (HLA-DR) gene expression is reduced in sepsis and correlates with impaired TNFα response: A diagnostic tool for immunosuppression? PLoS One 12, e0182427, 2017.

Wu B, Yang H, Ying S, Lu H, Wang W, Lv J, Xiong H, Hu W. High HLA-F expression is a poor prognosis factor in patients with nasopharyngeal carcinoma. Anal Cell Pathol (Amst) 2018, 7691704, 2018.

Wu C, Xu G, Tsai SA, Freed WJ, Lee CT. Transcriptional profiles of type 2 diabetes in human skeletal muscle reveal insulin resistance, metabolic defects, apoptosis, and molecular signatures of immune activation in response to infections. Biochem Biophys Res Commun 482, 282–288, 2017.

Xu Y, Han H, Zhang F, Lv S, Li Z, Fang Z. Lesion human leukocyte antigen-F expression is associated with a poor prognosis in patients with hepatocellular carcinoma. Oncol Lett 9, 300–304, 2013.

Yamaoka M, Maeda N, Nakamura S, Kashine S, Nakagawa Y, Hiuge-Shimizu A, Okita K, Imagawa A, Matsuzawa Y, Matsubara K, Funahashi T, Shimomura I. A pilot investigation of visceral fat adiposity and gene expression profile in peripheral blood cells. PLoS One 7, e47377, 2012.

Yamashita S, Fujii K, Zhao C, Takagi H, Katakura Y. Involvement of the NFX1-repressor complex in PKC-delta-induced repression of hTERT transcription. J Biochem 160, 309–313, 2016.

Yang YC, Chang TY, Chen TC, Lin WS, Chang SC, Lee YJ. Human leucocyte antigen-G polymorphisms are associated with cervical squamous cell carcinoma risk in Taiwanese women. Eur J Cancer 50, 469–474, 2014.

Yang Z, Li H, Wang Z, Yang Y, Niu J, Liu Y, Sun Z, Yin C. Microarray expression profile of long non-coding RNAs in human lung adenocarcinoma. Thorac Cancer 9, 1312–1322, 2018.

Yuzefovych LV, Musiyenko SI, Wilson GL, Rachek LI. Mitochondrial DNA damage and dysfunction, and oxidative stress are associated with endoplasmic reticulum stress, protein degradation and apoptosis in high fat diet-induced insulin resistance mice. PLoS One 8, e54059, 2013.

Zhang X, Lin A, Zhang JG, Bao WG, Xu DP, Ruan YY, Yan WH. Alteration of HLA-F and HLA I antigen expression in the tumor is associated with survival in patients with esophageal squamous cell carcinoma. Int J Cancer, 132, 82–89, 2013.

Zeestraten EC, Reimers MS, Saadatmand S, Dekker JW, Liefers GJ, van den Elsen PJ, van de Velde CJ, Kuppen PJ. Combined analysis of HLA class I, HLA-E and HLA-G predicts prognosis in colon cancer patients. Br J Cancer 110, 459–468, 2014.