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REVIEWS

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NUCLEAR RECEPTORS AND THEIR ROLE IN EPSTEIN — BARR VIRUS INDUCED B CELL TRANSFORMATION

*S.P. Yenamandra[1, 2], G. Klein[1], E. Kashuba[1, 2, 3, *]*

[1]*Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institute, Stockholm S17177, Sweden*

[2]*Center for Integrative Recognition in the Immune System (IRIS), Karolinska Institute, Stockholm S17177, Sweden*

[3]*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine, Kyiv 03022, Ukraine*

Epstein — Barr virus (EBV) is a lymphotropic virus that infects more than 90% of the human population, and targets B cells for infection. Infection of human B cells leads to the malignant transformation and eventual immortalization. In latency III infection six EBV-encoded nuclear antigens (EBNAs) and three latent membrane proteins (LMPs) are expressed in the transformed cells that can grow as a lymphoblastoid cell lines *in vitro*. These proteins hijack the normal B cell growth pathways by activating the constitutive growth promotion and external survival signals. We have determined a set of the nuclear receptors that are up- (and down-) regulated in the latency III infected cells at the mRNA level. In the present paper we discussed the possible role of these receptors in B cell transformation upon EBV infection based on the literature data.

Key Words: **cell transformation, EBV, EBNA, expression profiling, microarray, nuclear receptor.**

MAJOR FEATURES OF HERPESVIRUSES

Herpesviruses are often associated with different human diseases. One of the common features of all the members of the human herpesvirus (HHV) family is their ability to establish life-long persistence in the host after the primary infection. The latent infection by the human gamma (γ) herpesviruses, Epstein — Barr virus (EBV) and Kaposi's sarcoma virus (HHV8), could be established in lymphoid tissues. EBV, which was first identified in 1964, is a lymphotropic virus that infects more than 90% of the human population, and targets B cells for infection.

The primary infection may be asymptomatic and often occurs in early childhood. When the infection occurs later on in life, EBV causes infectious mononucleosis (IM). In immunosuppressed hosts, such as transplant recipients and AIDS patients, EBV may cause post-transplant lymphoproliferative disease (PTLD). EBV is also associated with several malignancies, such as Burkitt's lymphoma (BL), nasopharyngeal carcinoma (NPC), and Hodgkin's lymphoma (HL).

It is considered that the virus enters the host by infecting B cells, which infiltrate the epithelium of the oropharynx. After infection, the viral latent proteins are produced and the B cells turn into large immunoblasts that express six EBV-encoded nuclear antigens (EBNAs), three latent membrane proteins (LMPs) and two small non-polyadenylated RNAs (EBERs). Such cells may be targeted by cytotoxic T lymphocytes (CTLs) reacting with the latency associated viral antigens.

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*Correspondence: Fax: 468330498
E-mail: Elena.Kashuba@ki.se

Abbreviations used: BL – Burkitt lymphoma; CTL – cytotoxic T lymphocytes; EBER – EBV encoded small RNA; EBV – Epstein – Barr virus; EBNA – EBV-encoded nuclear antigen; HHV – human herpesvirus; HL – Hodgkin's lymphoma; LCL – lymphoblastoid cell line; LMP – latent membrane protein; NPC – nasopharyngeal carcinoma; PTLD – post-transplantation lymphoproliferative disease.

The activated cells differentiate into antibody producing cells, and migrate to the oropharyngeal mucosa. In some of them lytic gene expression is activated and new virus particles are produced. The virus is usually shed through the saliva of the infected host. The EBV-infected memory B cell pool may be enriched by re-entry of the latently infected cells into the germinal centers, where clonal expansion takes place (reviewed in [1, 2]).

INFECTION OF B CELLS WITH EBV *IN VITRO*

Infection of human B cells *in vitro* induces metabolic activation, morphological transformation, cell proliferation and eventual immortalization.

In EBV immortalized immunoblastic lines of non-neoplastic origin (lymphoblastoid cell lines; LCLs) nine viral proteins are expressed. Similar patterns of EBNAs and LMPs expression take place in tonsil and blood B cells of IM patients and in the lymphoblasts of individuals with PTLD [3–5]. EBV is the most efficient transforming tumor virus *in vitro* (reviewed in [6]). Six of the eleven EBV-encoded gene products (EBNA-1, -2, -3, -5, -6, LMP-1) are essential for EBV-mediated transformation [7]. The latency III genes hijack the normal B cell growth pathways by activating of the constitutive growth promotion and external survival signals.

EBV encodes EBNA-2 that activates and regulates the transcription of Notch and PU.1 responsive promoters of the cellular genes, such as *c-myc*, *c-fgr*, *CD21* and *CD23* promoters. EBNA-2 binding to RBP-Jκ results in the constitutive expression of *c-myc* in LCLs ([8], for review see [9]), leading to change in cell phenotype, adhesion and activation molecules expression. LMP1 activates TNFα/CD40 downstream signaling pathways that can stimulate cell growth and survival through activation of NFκB, jun and p38 MAPK (reviewed in [1]).

An aim of the present paper was to discuss the putative functions of the nuclear receptors that were

found to be differently expressed in the naïve and EBV-transformed B lymphocytes (see current issue, Yenamandra *et al.* [10]).

NUCLEAR RECEPTOR PROFILING

As it was demonstrated in [10], only 17 genes showed consistent differences in expression — decrease in B cells, upregulation in EBV-infected cells and LCLs, or *vice versa*. The expression of 5 genes was elevated in EBV-transformed cells; whereas 12 genes were downregulated in LCLs [10].

Nuclear receptors that were up-regulated in the EBV-transformed cells

Nr2F2. Nuclear receptor subfamily 2, group F, member 2. This receptor is also known as a transcriptional factor COUP2, or COUPTFII (**NP_066285**, OMIM *107773, gene is located on chromosome 15q26.1-26.2, protein consists of 414 amino acids). The literature data concerning this receptor is quite poor. However, it may play an important role in EBV induced transformation, because COUPTFII is involved in the repression of Notch signaling. It can also down-regulate the *Apolipoprotein 1* [11, 12] (Fig. 1, *a*).

Nr4A3. Nuclear receptor subfamily 4, group A, member 3. This protein is also called the Mitogen-induced orphan receptor, MINOR (**NP_775290**, OMIM +600542, gene is located on chromosome 9q22, protein is 626 amino acids long). It belongs to NUR77 family proteins. It was shown that LPS, cytokines, oxidized lipids, and low-density lipoproteins induced Nr4A1-3 in macrophages (reviewed in [13]). Overexpression of this protein may lead to the apoptosis of T cells. At certain conditions, MINOR supports the survival of cells (reviewed in [14, 15]) (Fig. 1, *b*). Nr4A3 can play an important role in the inflammatory signaling, because Nr4A receptors 1–3 are induced by inflammation (by NF-κB, for example) (see Fig. 1, *b*).

Nr6A1. Nuclear receptor subfamily 6, group A, member 6. This receptor has few alternative names — Germ cell nuclear factor (GCNF), and Retinoid receptor-related testis associated receptor (RTR) (**NP_201591**, OMIM *602778, gene is localized to chromosome 9q33-34.1, protein consists of 480 amino acids). It is quite well studied protein (reviewed in [16]). Recently, it was shown that loss of Nr6A1 expression led to induction of *Nanog*, *Oct4*, *Sox2*, *Stella* and *FGF4* expression upon retinoic acid (RA) treatment [17]. The other genes, such as *Fgf8*, *Wnt1*, *Pax2/5*, *En1/2*, and *Gbx2*, were downregulated in case of Nr6A1 expression loss [18]. An intriguing feature of this orphan receptor is ability to recruit DNA

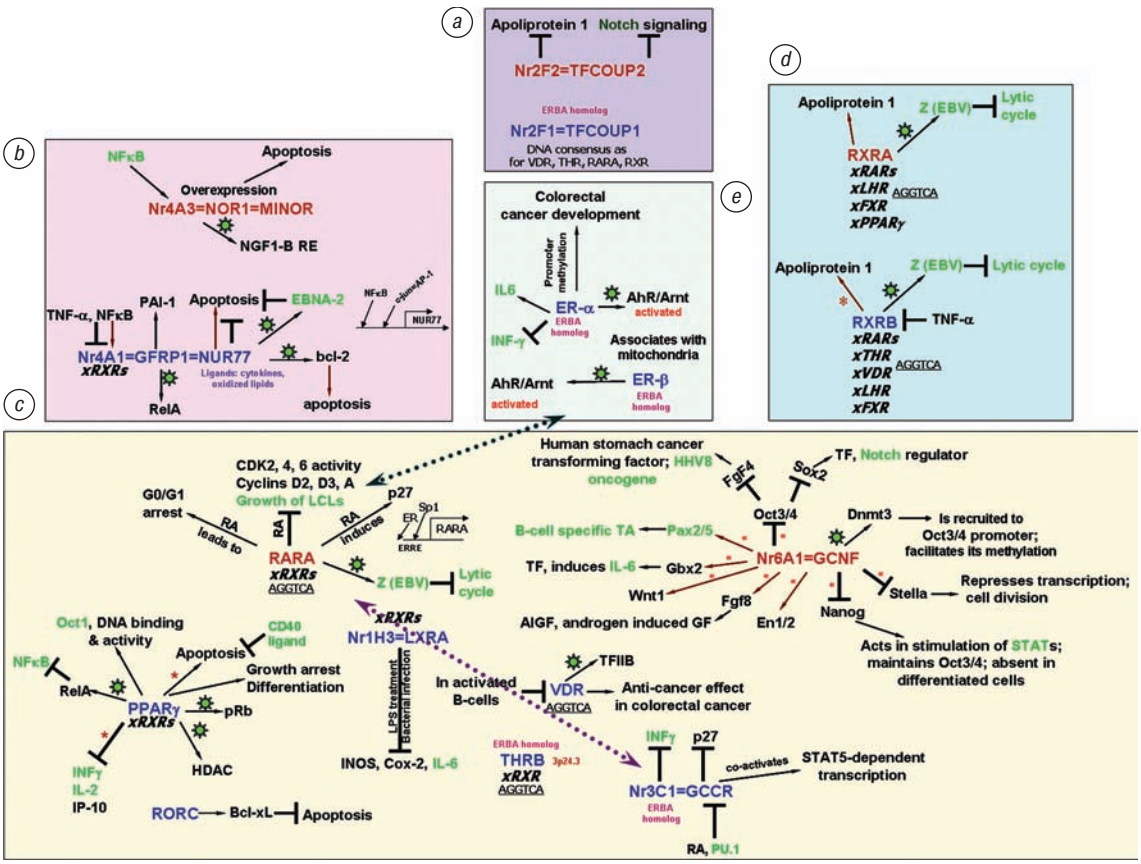

Fig. 1. *a* — Properties of cluster of Nr2F proteins. Nr2F2 down regulates Apoliprotein 1 and Notch signaling. The up-regulated receptors are shown is the red font; the down-regulated receptors are shown in blue. Sign "T" indicates inhibition of a process or a protein; stars — binding between proteins; text in green is related to EBV-field. *b* — Properties of cluster of NUR77 family proteins. NUR77 binds to RelA, bcl-2 and can induce apoptosis. EBNA-2 binds to NUR77 and blocks apoptosis. *c* — Properties of Nr641, PPARG and RARA nuclear receptors. Nr6A1 might be involved in the regulation of Notch signaling; PPARG can block NBκB pathway; RARA binds to Z promoter of EBV and can prevent the lytic cycle. *d* — Properties of RXR proteins. These proteins bind to Z promoter of EBV and prevent the induction of the lytic cycle. The up-regulated receptors are shown is the red font; the down-regulated receptors are shown in blue. *e* — Properties of estrogen receptors: the cross talk between ER pathway and Aryl hydrocarbon (dioxin) receptor (AhR) pathway

methyltransferase (Dnmt3) to the *Oct3/4* promoter, which resulted in silencing by hypermethylation [19] (Fig. 1, *c*).

RARA. Retinoic acid receptor α (**NP_000955**, OMIM *180240, gene is located on chromosome 17q21.1, protein consists of 462 amino acids) is very well studied receptor (reviewed in [20]). It binds to DNA as a homodimer with the Retinoid X receptors (RXRs). It was shown that activated RARA can block growth of LCLs due to G_0/G_1 arrest, while activity of CDK2, -4, and -6 was inhibited, as well as cyclin A, D2 and D3 [21].

On the other hand, it was reported that EBV lytic cycle activation was inhibited upon RA treatment due to the direct binding between RARA and the EBV-encoded lytic protein BZLF1 [22, 23].

Earlier it was also found that retinoids inhibited naïve B cell proliferation, but promoted cell survival [24]. It is worth to mention that the promoter region of RARA contains estrogen receptor response element (see Fig. 1, *c*) [25].

RXRA. Retinoid X receptor α (**NP_002948**, OMIM *180245, gene is located on chromosome 9q34.3, protein consists of 462 amino acids). A ligand-bound receptor can activate transcription as a homodimer or heterodimer (with RARs, LXR, FXR, PPARG) from responsive elements containing two degenerate copies of the consensus motif AGGTCA. The same sites are used by RARs, THRs, VDR and RXRs (Fig. 1, *d*).

It was shown (as for RARA), that RXRA could bind to the BZLF1 (Z-protein) and inhibit EBV lytic cycle [26, 27].

It was reported that RXR agonist treatment led to an activation of Bcl2a1 (BFL1) and, thus, decreased apoptosis in the naïve T-cells [26]. Moreover, recently it was found that EBNA-2 transactivated BFL1 through RBP-Jκ [27]. BFL1 was activated by CD40, TNF, IL-1, and NFκB as well.

Nuclear receptors that were down-regulated in the EBV-transformed cells

PPAR-γ. Peroxisome proliferator-activated receptor γ, also known as PPARG (**NP_619725**, OMIM *601487, gene is located on chromosome 3p21, protein consists of 475 amino acids) forms dimer with RXRs to activate transcription. It was shown that PPARG might function as a promoter-specific repressor of NF-κB target genes that regulate immunity and homeostasis [28]. PPARG binds to Rel A [29]. Decrease in PPAR-γ abolishes the nuclear export of RelA.

It was already shown that lymphocytes expressed functioning PPARG, and its activation led to apoptosis, or growth arrest [30, 31], or differentiation [32]. Interestingly, the activation of PPARG in B cells by CD40 ligand protects spleen B cells and B cell lymphomas tumor cells from apoptosis [33].

Another attractive feature of PPARG is the ability to bind pRb and histone deacetylase 3 (HDAC3) [34]. By binding to HDAC3, PPARG can induce cell cycle arrest of pRb positive cells in G_1 phase.

PPARG, as we mentioned above, can have both transactivating and transrepressing activity [35]. PPARG can repress some IFN-γ and LPS-inducible genes, such as IL-12 and IP10 (see Fig. 1, *c*). In their turn, cytokines can repress the activity of PPARG by inhibiting DNA binding [36].

ER-α. Estrogen receptor α, ESR (**NP_000116**, OMIM *133430, gene is located on chromosome 6q25.1, 595 amino acids in the protein sequence). It was shown that promoter methylation decreased the level of ESR expression in colorectal tumors [37]. Recently, it was demonstrated that Arnt/AhR heterodimer could bind to ESR1 (both, α and β). This multiprotein complex (in the presence of p300) activates transcription from ER responsive elements (ERRE) [38] (Fig. 1, *e*).

It was shown that ER-α and -β are expressed in peripheral blood B cells. B cells express more ER-β than ER-α [39]. However, only ER-α is needed to upregulate immunoglobulin production. Both activated forms are required for complete downregulation of lymphopoiesis in the bone marrow of mice [40, 41].

ER-β receptor is known also as ER2, ESR2 (**Q92731**, OMIM *601663, gene is localized to chromosome 14q, protein consists of 447 amino acids). ER-β putative DNA-binding domain shows 96% identity to that of ER-α, but the ligand-binding domain has much lower homology — only 56%. Actually, binding of ER-α to DNA and transcription of the ER-α dependent genes are studied very well, but ER-β function is poorly known. It was published recently that ER-β was associated with mitochondria [42]. Interestingly, that treatment of mice by ER-α selective agonist led to the decrease in number of mature B cells, and to the enhancement of INF-γ production and IL-6 suppression [43]. The most intriguing fact is that a disruption of ER-β in mice led to myeloproliferative disease that resembled chronic myeloid leukemia with lymphoid blast crisis [44] (see Fig. 1, *e*).

Nr1H3. Nuclear receptor subfamily 1, group H, member 3, also known as Liver X receptor α (LXRA) (**NP_005684**, OMIM *602423, gene is localized to chromosome 11p12, protein consists of 447 amino acids). This protein binds to RXRs and to DNA as heteromider [45]. LXRs play important role in the lipid metabolism and transport. It was shown that LXR ligands could inhibit expression of the inflammatory regulators, such, as INOS, COX-2, and IL6 in response to bacterial infection or LPS stimulation [46]. Moreover, LXRs-null mice are highly susceptible to infection with Listeria monocytogenes [47] (see Fig. 1, *c*).

Nr2F1. Nuclear receptor subfamily 2, group F, member 1, also known as transcription factor COUP1 (TFCOUP1) (**NP_005645**, OMIM *132890, gene is located on chromosome 5q14, protein consists of 423 amino acids). It was shown that Nr2F1 recognizes DNA consensus sites that are also sites for RARA, RXRs, VDR, and THR. The receptor shows high homology to v-ErbA protein. Interestingly that compared with naïve B-cells, BLs, and LCLs, transcription factor Nr2F1

was up-regulated in Reed/Sternberg cells of HL [48]. Unfortunately, there are only few studies devoted to Nr2F1 (see Fig. 1, *a*).

Nr3C1. Nuclear receptor subfamily 3, group C, member 1. This receptor is known as glucocorticoid receptor (GCCR) (**NP_001018087**, OMIM +138040, gene is localized to chromosome 5q31, protein consists of 777 amino acids). About two decades ago it was shown that GCCR might enhance the B cell maturation and immunoglobulin production [49, 50]. An attempt to measure the concentration and activity of receptor in peripheral blood B cells and EBV-infected B cells was carried out [51]. However, no difference in the GCCR activity was observed. It was shown later on, that LPS-treated B cells are more resistant to the apoptosis, mediated by activated GCCR [52].

Interestingly, the activated GCCR induced CD40 mRNA [53] and could inhibit growth arrest due to RA treatment [54]. The promoter of GCCR is down regulated by PU.1 [55].

It was shown recently that endogenous glucocorticoids are required for transcriptional suppression of INF-γ [56]. At the same time, activated GCCR acts as a co-activator for STAT5-dependent transcription [57] (see Fig. 1, *c*).

Nr4A1. Nuclear receptor subfamily 4, group A, member 1, homologues of mouse NUR77 (**NP_775180**, OMIM *139139, gene is localized to chromosome 12q13, protein consists of 598 amino acids). It was shown that this receptor was induced rapidly and transiently by growth-stimulating agents in human lymphocytes [58]. LPS treatment of macrophages led to N4A1 induction by NF-κB [59]. In contrast to transcriptionally active nuclear localization [60], Nr4A1 receptor exhibits its mitogenic effect through the target gene regulation. Its pro-apoptotic effect is realized in cytoplasm through regulation of mitochondrial activity [61]. It was found later that the orphan receptor is bound to Bcl-2 and could convert it to killer [62].

It should be mentioned that at different conditions and cell background NUR77 could be both induced and repressed by TNF-α and NF-κB [60, 63, 64]. NUR77 was implicated in B cell apoptosis [65], and EBNA-2 (but not LMP1) could protect B cells from NUR77-induced apoptosis [66, 67] (see Fig. 1, *b*).

RORC. RAR-related orphan receptor γ (RORG) (**NP_005051**, OMIM *602943, gene is located on chromosome 1q21, protein contains 518 amino acids). Importance of RORC was shown in experiments on the homozygous null mice — they lacked peripheral and mesenteric lymphnodes and Peyer patches [68, 69]. Another interesting feature of such mice is loss of thymic expression of the anti-apoptotic factor Bcl-xL [68, 70] (see Fig. 1, *c*).

RXRB. Retinoid X receptor β (**NP_068811**, OMIM *180246, gene is localized to chromosome 6p21.3, protein consists of 533 amino acids). This receptor acts as transcription activator in the heterodimer with RARs, VDR, THR, LXR, and Farnesoid X receptor (FXR).

RXRB increases their DNA binding and transactivating ability of RARs, VDR, and THR [71, 72]. RXRB promoter is down-regulated by TNF-α and this repression is mediated by p38 MAP kinase, independently from NF-κB [73]. RXRB binds to Z protein as RXRA does [22, 23] and this binding inhibits the EBV lytic cycle (see Fig. 1, *d*).

THRB. Thyroid hormone receptor β (**NP_000452**, OMIM +190160, gene is located on chromosome 3p24.3, protein consists of 461 amino acids). This receptor was known before as ERBA-2, or human homologue of retroviral ERBA protein (avian erytroblastic leukemia viral oncogene). Interestingly, homozygous knock-out mice had an elevated level of thyroid-stimulating hormone [74, 75]. However, the responsible genes for THRB remain largely unknown (see Fig. 1, *c*).

VDR. Vitamin D3 receptor (**NP_000367**, OMIM *601769, gene is localized to chromosome 12q12-14, protein consists of 427 amino acids). VDR possesses Zn-finger in its N-terminal domain. This part of protein binds to general transcription factor II B (TFIIB) [76].

It was shown quite long ago that the VDR-dependent gene regulation is blocked in B cells (peripheral blood cells and LCLs) [77]. The active VDR pathway could inhibit proliferation and enhance differentiation of leukemic cells [78]. The level of VDR expression (at mRNA and protein levels) was lower in the EBV transformed cells compared to tonsil B cells [78].

Recently an anti-tumor effect was proposed for vitamin D and VDR (for review see [79, 80]) (see Fig. 1, *c*).

CLUSTERS OF NUCLEAR RECEPTORS

We have analyzed the targeted nuclear receptors, and grouped them into clusters. A phylogram is presented on Fig. 2.

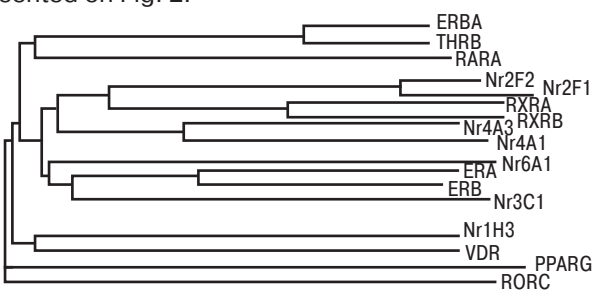

Fig. 2. Phylogram of 16 nuclear receptors that expressed differently in naïve and EBV-infected B-cells

Cluster of Nr2F proteins (COUP transcription factors). These two proteins are the most closely related among all of the discussed receptors. The alignment score is 87 (Clustal W). Nr2F1 is downregulated but Nr2F2 is upregulated. Unfortunately, not much is known about these receptors. It is only known that Nr2F2 is involved in Notch signaling repression (see Fig. 1, *a*). It is important to continue the study on Nr2F2 receptor, because Notch pathway is used by EBV upon latency establishing: EBNA-2 and EBNA-3 family proteins regulate the expression of Notch-dependent genes.

Cluster of RXR proteins. These two receptors have alignment score of 69, it is just little lower than for THR and v-ERBA-77 (see the phylogenetic tree, Fig. 2).

The most interesting their feature is the ability to bind Z-protein of EBV and inhibit its transcriptional activity, i.e. prevent reactivation of the lytic cycle (see Fig. 1, *d*). That makes RXR proteins the important players in the control on latency.

Cluster of NUR 77 family proteins. These proteins showed quite high alignment score — 47. Interestingly, NF-κB activates NOR1 and can both downregulate and upregulate NUR77 transcription (see Fig. 1, *b*). It was shown earlier that both receptors can regulate apoptosis. However, the cell fate depends on the delicate balance between them. EBNA-2 binds to NUR77, and this binding blocks apoptosis [66, 67].

Cluster of estrogen receptors. The alignment score for ER-α and ER-β is not very high — 44. In contrast to the first two clusters, both estrogen receptors (α and β) are downregulated in the EBV-infected cells and LCLs (see Fig. 1, *e*). No data on EBV-infected cells were reported yet.

Both ERs can be regulated by AhR/Arnt heterodimer in the absence of ligand, which is the fascinating link to the role of ERs in the EBV induced transformation. Recently, we have shown that EBNA-3 could bind AhR and the nuclear fraction of AhR was enriched in the presence of EBNA-3 [81]. It is likely that EBNA-3 family proteins may interfere with ERs. This hypothesis should be further elucidated.

Next cluster includes all of other receptors (see Fig. 1, *c*). All of the 16 proteins discussed here share common features — they possess C4 zinc finger of nuclear hormone receptor (**smart 00399**, ZnF_C4) and the ligand-binding domain of nuclear receptors (**smart 00430**, HOLI). All of them are homologues to v-ERBA with different score of alignment (highest — THR, score of 77; lowest — GCCR, score of 16).

THE INTERSECTION OF NUCLEAR RECEPTOR PATHWAYS

As we have already mentioned, we have determined a set of nuclear receptors that are up- (and down-) regulated in the latency III infected cells [10]. Some receptors were shown before to be implicated in the EBV biology, like NUR77, RXRA, RXRB, RARA, and GCCR. Other proteins were not connected to lymphoblasts — MINOR, TF COUP I and II, ER-α and -β, PPARG, RORC, LXRA, THRB, VDR, and GCNF. The important role of the regulation of the expression of nuclear receptors in transformation process may be concluded from the fact that many of them can control transcription of genes involved in B cell activation or apoptosis. For example, upregulated GCNF gene can activate B cell specific transcription factors Pax2/5 and Gbx2 that specifically induce IL-6 [17, 18].

We have to mention that data obtained in our study [10] show a correlation with the literature data. For instance, others [30, 33] and we [10] have shown that CD40 ligand downregulated PPARG, which can induce apoptosis in B cells. Importantly, PPARG was downregulated in the EBV-infected cells and LCLs [10]. Moreover, not only protein level was changed, but a cellular distribution of PPARG in the naïve, activated and infected cells as well. As we discussed earlier, the NFκB pathway becomes activated upon EBV-induced transformation. From the other hand, it was reported that PPARG binding to RelA resulted in the nuclear export of RelA, thus inhibiting NF-κB pathway [31]. Noteworthy, EBV decreased mRNA level of PPARG and changed the cellular distribution of PPARG [10].

It is important to notice that the ERs (both, -α and -β) have an ERRE in the promoter region of RARA gene. This makes them an important target to study in the process of EBV-induced transformation.

Also, we have found that there is a crosstalk between RARA and GCCR. RARA binds the Z lytic protein of EBV, and this binding prevents re-activation of the lytic cycle in the latency III cells. RXRA and RXRB bind Z protein too: one of them is upregulated and other is downregulated by EBV (see Fig. 1). Probably, EBV delicately regulates the levels of these receptors to achieve the optimal expression of the target genes involved in the B cell transformation.

Summarizing, we can conclude that there is a wide unexplored area of the role of nuclear receptors that might be involved in EBV-induced B cell transformation. Extensive study of cellular nuclear receptor pathways is needed to fully understand their role in the process of malignant cell transformation.

REFERENCES

1. **Kieff E, Rikinson A.** Epstein — Barr virus and its replication. In: Fields BN, Knipe DM, Howley PM, et al, eds. Fields Virology. Philadelphia: Lippincott Williams&Wilkins, 2001: 2511–74.

2. **Rikinson A, Kieff E.** Epstein — Barr virus. In: Fields BN, Knipe DM, Howley PM, et al, eds. Fields Virology. Philadelphia: Lippincott Williams&Wilkins, 2001: 2575–628.

3. **Hopwood PA, Brooks L, Parratt R, *et al.*** Persistent epstein — Barr virus infection: unrestricted latent and lytic viral gene expression in healthy immunosuppressed transplant recipients. Transplantation 2002; **74**: 194–202.

4. **Joseph AM, Babcock GJ, Thorley-Lawson DA.** Cells expressing the Epstein — Barr virus growth program are present in and restricted to the naive B-cell subset of healthy tonsils. J Virol 2000; **74**: 9964–71.

5. **Joseph AM, Babcock GJ, Thorley-Lawson DA.** EBV persistence involves strict selection of latently infected B cells. J Immunol 2000; **165**: 2975–81.

6. **Klein G.** Perspectives in studies of human tumor viruses. Front Biosci 2002; **7**: d268–74.

7. **Tomkinson B, Robertson E, Kieff E.** Epstein — Barr virus nuclear proteins EBNA-3A and EBNA-3C are essential for B-lymphocyte growth transformation. J Virol 1993; **67**: 2014–25.

8. **Kaiser C, Laux G, Eick D, *et al.*** The proto-oncogene c-myc is a direct target gene of Epstein — Barr virus nuclear antigen 2. J Virol 1999; **73**: 4481–4.

9. **Bornkamm GW, Hammerschmidt W.** Molecular virology of Epstein — Barr virus. Philos Trans R Soc Lond B Biol Sci 2001; **356**: 37–59.

10. **Yenamandra SP, Lundin A, Arulampalam V, *et al.*** Expression profile of nuclear receptors upon Epstein — Barr virus induced B-cell transformation. Exp Oncol 2009; **31**: 92–6.

11. **You LR, Lin FJ, Lee CT, *et al.*** Suppression of Notch signalling by the COUP-TFII transcription factor regulates vein identity. Nature 2005; **435**: 98–104.

12. **You LR, Takamoto N, Yu CT, *et al.*** Mouse lacking COUP-TFII as an animal model of Bochdalek-type congenital diaphragmatic hernia. Proc Natl Acad Sci USA 2005; **102**: 16351–6.

13. **Winoto A, and Littman DR.** Nuclear hormone receptors in T lymphocytes. Cell 2002; **109 Suppl**: S57–66.

14. **He YW.** Orphan nuclear receptors in T lymphocyte development. J Leukoc Biol 2002; **72**: 440–6.

15. **Li QX, Ke N, Sundaram R, *et al.*** NR4A1, 2, 3 — an orphan nuclear hormone receptor family involved in cell apoptosis and carcinogenesis. Histol Histopathol 2006; **21**: 33–40.

16. **Zechel C.** The germ cell nuclear factor (GCNF). Mol Reprod Dev 2005; **72**: 550–6.

17. **Gu P, LeMenuet D, Chung AC, *et al.*** Orphan nuclear receptor GCNF is required for the repression of pluripotency genes during retinoic acid-induced embryonic stem cell differentiation. Mol Cell Biol 2005; **25**: 8507–19.

18. **Chung AC, Xu X, Niederreither KA, *et al.*** Cooney, Loss of orphan nuclear receptor GCNF function disrupts forebrain development and the establishment of the isthmic organizer. Dev Biol 2006; **293**: 13–24.

19. **Sato N, Kondo M, Arai K.** The orphan nuclear receptor GCNF recruits DNA methyltransferase for Oct-3/4 silencing. Biochem Biophys Res Commun 2006; **344**: 845–51.

20. **Mattson JC.** Acute promyelocytic leukemia. From morphology to molecular lesions. Clin Lab Med 2000; **20**: 83–103.

21. **Cariati R, Zancai P, Quaia M, *et al.*** Retinoic acid induces persistent, RARalpha-mediated anti-proliferative responses in Epstein-Barr virus-immortalized b lymphoblasts carrying an activated C-MYC oncogene but not in Burkitt's lymphoma cell lines. Int J Cancer 2000; **86**: 375–84.

22. **Sista ND, Pagano JS, Liao W, *et al.*** Retinoic acid is a negative regulator of the Epstein — Barr virus protein (BZLF1) that mediates disruption of latent infection. PROC NATL ACAD SCI USA 1993; **90**: 3894–8.

23. **Sista ND, Barry C, Sampson K, *et al.*** Physical and functional interaction of the Epstein-Barr virus BZLF1 transactivator with the retinoic acid receptors RAR alpha and RXR alpha. Nucleic Acids Res 1995; **23**: 1729–36.

24. **Lomo J, Smeland EB, Ulven S, *et al.*** RAR-, not RXR, ligands inhibit cell activation and prevent apoptosis in B-lymphocytes. J Cell Physiol 1998; **175**: 68–77.

25. **Laganiere J, Deblois G, Giguere V.** Functional genomics identifies a mechanism for estrogen activation of the retinoic acid receptor alpha1 gene in breast cancer cells. Mol Endocrinol 2005; **19**: 1584–92.

26. **Rasooly R, Schuster GU, Gregg JP, *et al.*** Retinoid x receptor agonists increase bcl2a1 expression and decrease apoptosis of naive T lymphocytes. J Immunol 2005; **175**: 7916–29.

27. **Pegman PM, Smith SM, D'Souza BN, *et al.*** Epstein — Barr virus nuclear antigen 2 trans-activates the cellular anti-apoptotic bfl-1 gene by a CBF1/RBPJ κ-dependent pathway. J Virol 2006; **80**: 8133–44.

28. **Pascual G, Fong AL, Ogawa S, *et al.*** A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. Nature 2005; **437**: 759–63.

29. **Kelly D, Campbell JI, King TP, *et al.*** G Grant, EA Jansson, AG Coutts, S Pettersson, and S Conway, Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol 2004; **5**: 104–12.

30. **Jones DC, Ding X, Daynes RA.** Nuclear receptor peroxisome proliferator-activated receptor alpha (PPARalpha) is expressed in resting murine lymphocytes. The PPARalpha in T and B lymphocytes is both transactivation and transrepression competent. J Biol Chem 2002; **277**: 6838–45.

31. **Schlezinger JJ, Jensen BA, Mann KK, *et al.*** Peroxisome proliferator-activated receptor gamma-mediated NF-κB activation and apoptosis in pre-B cells. J Immunol 2002; **169**: 6831–41.

32. **Konopleva M, Elstner E, McQueen TJ, *et al.*** Peroxisome proliferator-activated receptor gamma and retinoid X receptor ligands are potent inducers of differentiation and apoptosis in leukemias. Mol Cancer Ther 2004; **3**: 1249–62.

33. **Ray DM, Akbiyik F, Bernstein SH, *et al.*** CD40 engagement prevents peroxisome proliferator-activated receptor gamma agonist-induced apoptosis of B lymphocytes and B lymphoma cells by an NF-kappa B-dependent mechanism. J Immunol 2005; **174**: 4060–9.

34. **Fajas L, Egler V, Reiter R, *et al.*** PPARgamma controls cell proliferation and apoptosis in an RB-dependent manner. Oncogene 2003; **22**: 4186–93.

35. **Welch JS, Ricote M, Akiyama TE, *et al.*** PPARgamma and PPARdelta negatively regulate specific subsets of lipopolysaccharide and IFN-gamma target genes in macrophages. Proc Natl Acad Sci USA 2003; **100**: 6712–7.

36. **Suzawa M, Takada I, Yanagisawa J, *et al.*** Cytokines suppress adipogenesis and PPAR-gamma function through the TAK1/TAB1/NIK cascade. Nat Cell Biol 2003; **5**: 24–30.

37. **Issa JP, Ottaviano YL, Celano P, *et al.*** Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. Nat Genet 1994; **7**: 536–40.

38. **Ohtake F, Takeyama K, Matsumoto T, *et al.*** Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. Nature 2003; **423**: 545–50.

39. **Phiel KL, Henderson RA, Adelman SJ, *et al.*** Elloso, Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. Immunol Lett 2005; **97**: 107–13.

40. **Erlandsson MC, Jonsson C.A, Islander U, *et al.*** Oestrogen receptor specificity in oestradiol-mediated effects on B lymphopoiesis and immunoglobulin production in male mice. Immunology 2003; **108**: 46–51.

41. **Islander U, Erlandsson MC, Hasseus B, *et al.*** Influence of oestrogen receptor alpha and β on the immune system in aged female mice. Immunology 2003; **110**: 149–57.

42. **Yang SH, Liu R, Perez EJ, *et al.*** Mitochondrial localization of estrogen receptor β. Proc Natl Acad Sci USA 2004; **101**: 4130–5.

43. **Li J, McMurray RW.** Effects of estrogen receptor subtype-selective agonists on immune functions in ovariectomized mice. Int Immunopharmacol 2006; **6**: 1413–23.

44. **Shim GJ, Wang L, Andersson S, *et al.*** Disruption of the estrogen receptor beta gene in mice causes myeloproliferative disease resembling chronic myeloid leukemia with lymphoid blast crisis. Proc Natl Acad Sci USA 2003; **100**: 6694–9.

45. **Willy PJ, Umesono K, Ong ES, *et al.*** LXR, a nuclear receptor that defines a distinct retinoid response pathway. Genes Dev 1995; **9**: 1033–45.

46. **Joseph SB, Castrillo A, Laffitte BA, *et al.*** Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. Nat Med 2003; **9**: 213–9.

47. **Joseph SB, Bradley MN, Castrillo A, *et al.*** LXR-dependent gene expression is important for macrophage survival and the innate immune response. Cell 2004; **119**: 299–309.

48. **Kuppers R, Klein U, Schwering I, *et al.*** Identification of Hodgkin and Reed-Sternberg cell-specific genes by gene expression profiling. J Clin Invest 2003; **111**: 529–37.

49. **Grayson J, Dooley NJ, Koski IR, *et al.*** Immunoglobulin production induced in vitro by glucocorticoid hormones:

T cell-dependent stimulation of immunoglobulin production without B cell proliferation in cultures of human peripheral blood lymphocytes. J Clin Invest 1981; **68**: 1539–47.

50. **Emilie D, Karray B, Crevon MC, *et al.*** B cell differentiation and interleukin 2 (IL2): corticosteroids interact with monocytes to enhance the effect of IL2. Eur J Immunol 1987; **17**: 791–5.

51. **Tomita M, Chrousos GP, Brandon DD, *et al.*** Glucocorticoid receptors in Epstein — Barr virus-transformed human lymphocytes. Horm Metab Res 1985; **17**: 674–8.

52. **Andreau K, Lemaire C, Souvannavong V, *et al.*** Induction of apoptosis by dexamethasone in the B cell lineage. Immunopharmacology 1998; **40**: 67–76.

53. **Jabara HH, Brodeur SR, Geha RS.** Glucocorticoids upregulate CD40 ligand expression and induce CD40L-dependent immunoglobulin isotype switching. J Clin Invest 2001; **107**: 371–8.

54. **Quaia M, Zancai P, Cariati R, *et al.*** Glucocorticoids promote the proliferation and antagonize the retinoic acid-mediated growth suppression of Epstein-Barr virus-immortalized B lymphocytes. Blood 2000; **96**: 711–8.

55. **Geng CD, Vedeckis WV.** c-Myb and members of the c-Ets family of transcription factors act as molecular switches to mediate opposite steroid regulation of the human glucocorticoid receptor 1A promoter. J Biol Chem 2005; **280**: 43264–71.

56. **Brewer JA, Khor B, Vogt SK, *et al.*** T-cell glucocorticoid receptor is required to suppress COX-2-mediated lethal immune activation. Nat Med 2003; **9**: 1318–22.

57. **Tronche F, Opherk C, Moriggl R, *et al.*** Glucocorticoid receptor function in hepatocytes is essential to promote postnatal body growth. Genes Dev 2004; **18**: 492–7.

58. **Nakai A, Kartha S, Sakurai A, *et al.*** A human early response gene homologous to murine nur77 and rat NGFI-B, and related to the nuclear receptor superfamily. Mol Endocrinol 1990; **4**: 1438–43.

59. **Pei L, Castrillo A, and Tontonoz P.** Regulation of macrophage inflammatory gene expression by the orphan nuclear receptor Nur77. Mol Endocrinol 2006; **20**: 786–94.

60. **Pei L, Castrillo A, Chen M, *et al.*** Induction of NR4A orphan nuclear receptor expression in macrophages in response to inflammatory stimuli. J Biol Chem 2005; **280**: 29256–62.

61. **Li H, Kolluri SK, Gu J, *et al.*** Cytochrome c release and apoptosis induced by mitochondrial targeting of nuclear orphan receptor TR3. Science 2000; **289**: 1159–64.

62. **Lin B, Kolluri SK, Lin F, *et al.*** Conversion of Bcl-2 from protector to killer by interaction with nuclear orphan receptor Nur77/TR3. Cell 2004; **116**: 527–40.

63. **Suzuki S, Suzuki N, Mirtsos C, *et al.*** Nur77 as a survival factor in tumor necrosis factor signaling. Proc Natl Acad Sci USA 2003; **100**: 8276–80.

64. **Hong CY, Park JH, Ahn RS, *et al.*** Molecular mechanism of suppression of testicular steroidogenesis by proinflammatory cytokine tumor necrosis factor alpha. Mol Cell Biol 2004; **24**: 2593–604.

65. **Mapara MY, Weinmann P, Bommert K, *et al.*** Involvement of NAK-1, the human nur77 homologue, in surface IgM-mediated apoptosis in Burkitt lymphoma cell line BL41. Eur J Immunol 1995; **25**: 2506–10.

66. **Lee JM, Lee KH, Weidner M, *et al.*** Epstein — Barr virus EBNA2 blocks Nur77-mediated apoptosis. Proc Natl Acad Sci USA 2002; **99**: 11878–83.

67. **Lee JM, Lee KH, Farrell CJ, *et al.*** EBNA2 is required for protection of latently Epstein-Barr virus-infected B cells against specific apoptotic stimuli. J Virol 2004; **78**: 12694–7.

68. **Kurebayashi S, Ueda E, Sakaue M, *et al.*** Retinoid-related orphan receptor gamma (RORgamma) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis. Proc Natl Acad Sci USA 2000; **97**: 10132–7.

69. **Eberl G, Marmon S, Sunshine MJ, *et al.*** An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells. Nat Immunol 2004; **5**: 64–73.

70. **Sun Z, Unutmaz D, Zou YR, *et al.*** Requirement for RORgamma in thymocyte survival and lymphoid organ development. Science 2000; **288**: 2369–73.

71. **Yu VC, Delsert C, Andersen B, *et al.*** RXR beta: a coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. Cell 1991; **67**: 1251–66.

72. **Hallenbeck PL, Marks MS, Lippoldt E, *et al.*** Heterodimerization of thyroid hormone (TH) receptor with H-2RIIBP (RXR beta) enhances DNA binding and TH-dependent transcriptional activation. Proc Natl Acad Sci USA1992; **89**: 5572–6.

73. **Sugawara A, Uruno A, Nagata T, *et al.*** Characterization of mouse retinoid X receptor (RXR)-beta gene promoter: negative regulation by tumor necrosis factor (TNF)-alpha. Endocrinology 1998; **139**: 3030–3.

74. **Forrest D, Erway LC, Ng L, *et al.*** Thyroid hormone receptor beta is essential for development of auditory function. Nat Genet 1996; **13**: 354–7.

75. **Forrest D, Hanebuth E, Smeyne RJ, *et al.*** Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor beta: evidence for tissue-specific modulation of receptor function. Embo J 1996; **15**: 3006–15.

76. **Jurutka PW, Remus LS, Whitfield GK, *et al.*** The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. Mol Endocrinol 2000; **14**: 401–20.

77. **Morgan JW, Reddy GS, Uskokovic MR, *et al.*** Functional block for 1 alpha,25-dihydroxyvitamin D3-mediated gene regulation in human B lymphocytes. J Biol Chem 1994; **269**: 13437–43.

78. **Elstner E, Lee YY, Hashiya M, *et al.*** 1 alpha,25-Dihydroxy-20-epi-vitamin D3: an extraordinarily potent inhibitor of leukemic cell growth in vitro. Blood 1994; **84**: 1960–7.

79. **Gombart AF, Luong QT, and Koeffler HP.** Vitamin D compounds: activity against microbes and cancer. Anticancer Res 2006; **26**: 2531–42.

80. **Norman AW.** Vitamin D Receptor (VDR): New assignments for an already busy receptor. Endocrinology 2006; **147**: 5542–8.

81. **Kashuba EV, Gradin K, Isaguliants M, *et al.*** Regulation of transactivation function of the aryl hydrocarbon receptor by the Epstein — Barr virus-encoded EBNA-3 protein. J Biol Chem 2006; **281**: 1215–23.