Genes Reprogramming During ATRA-induced Differentiation of Acute Promyelocytic Leukemia Cells

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Abstract: The therapeutic and preventive activities of retinoids in cancer are due to their ability to modulate the growth, differentiation, and survival or apoptosis of cancer cell. An acute promyelocytic leukemia cell line AP-1060 presents an abrupt response to all-trans retinoic acid (ATRA) which depends on its mutant PML/RARα. Microarray analysis of 9265 sequences demonstrated a complex cascade of reprogramming of AP1060 upon treatment with ATRA characterized by the differential expression of gene sets between induced (100nM ATRA) and sub-induced (10nM ATRA) cells. Among 1550 modulated genes by ATRA, 47% shown differential expression. A number of the small G-protein family was extensively involved in this reprogramming. The early up-regulated IL1 and down-regulated its angonistor may initiate the apoptosis pathway mediated by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Seven in absentia (Drosphila) homologies (SIAH-1, 2, and 3) considered as the inducers of apoptosis were repressed. Thus, a balanced functional network seem emerge and contribute to ATRA inducing differentiation based on the uniquely biologic and molecular characters of AP1060.
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1. Introduction

Acute promyelocytic leukemia (APL) is an interesting system in the study of human cancer because it is sole human malignancy that can be successfully treated with the induced differentiation therapy. The most of APL are characterized by the chromosome translocation t(15;17), and the fused PML/RARα resulted from this translocation is responsible for the leukemogenesis. It used to be believed that PML/RARα as a negative dominant factor abrupt PML and RXR by hterodimerization with PML/RARα (Raelson, 1996), which respectively interferes the function of RXR and PML for growth inhibition and apoptosis regulation and the differentiation pathway dependent on RXR (Kastner 1992; Grignani 1993; Rousselot 1994; Wang 1998). The early studies implies that the response of APL to ATRA base on the intrinsic molecular target PML/RARα to fusion gene which involved the gene transcriptional events that initiate the differentiation (Nervi and Nason-Burchenal 1998).

Both RARα and PML/RARα as interact with nuclear corepressor (N-CoR), mSin3 and a histone deacetylase (HAD) to form co-repressor complex: RARα-CoR or/and PML/RARα-CoR, the later is more stable (Dyck and Weis 1994; Lin and Minucci 2000). Deacetylation of histones by this complex may result in chromatin assembly and transcriptional repression. The exposure to ATRA orchestrates an ordered response of this apparatus involving initial dissociation of co-repressor from RARα or the RARα-portion of PML/RARα, but not from PLZF/RARα (an APL phenotype resistant to ATRA) (Dyck and Weis 1994), which permit association of co-activator (CoA) for forming a co-activator complex (CBP/P300, P/CAF, NcoA-1/SRC-1, P/CIP, and ACTR) to relieve the negative repression through post-transcriptional down-regulation of PML/RARα. The functional significance of histone deacetylase recruitment in APL pathogenesis was demonstrated by ability of histone deacetylase inhibitors in combination with ATRA to overcome the repressor activity of PML/RARα and PLZF/RARα in transient transfection assays, and to enhance the terminal differentiation of APL cells (Alland and Hong 1997; Lin , Grignani and Guidez 1998). In addition, recent evidence suggests that PML-RARα is also able to recruite the methylating enzymes (DnmT1 and Dnmt3a), leading to the hypermethylation of the RA target promoters resulting in transcriptional repression (Di Croce 2002; Villa 2006). These studies suggested that differentiation of APL by ATRA is triggered directly through a PML/RARα dependent signaling pathway.

We developed a new APL cell line AP1060 (Sun 2004), which harbored a missense mutation (Pro407Ser) in PML/RARα and presented an additional translocation t(3; 14) in addition to t(15;17). The very abrupt ATRA concentration response of AP-1060 may make this cell line of unusual value for analyzing subsets of response

genes. For example, evidence was presented that some genes, including CD18, CD38 and myeloblastin, can be turned on by ATRA (Shao 1997). This suggests that some genes are activated from normal RARα-responsive loci that are not susceptible to dominant negative repression by PML/RARα or possibly that some non-RARα mechanism is involved (Kang 1999). We undertook a study base on large-scale expression analysis, microarray to identify gene expression patterns in APL cells upon treatment with ATRA.

2. Material and Methods

Cell culture: AP-1060 cells were maintained in IMDM containing 10% FBS and 10% agar leukocyte conditioned medium (ALCM, StemCell Technologies, Vancouver, BC). For induced differentiation, cells at $1X10^6$ were suspended in IMDM containing 10% FBS supplemented with 5% ALCM, and treated by addition to the culture medium of either 10 nM, 100nM ATRA (Sigma, St. louis, MO). Control cells were treated stimultaneously carrier, and for each time point, a control flask was maitained for the same length of time and harvested in parallel.

Evaluation of the cellular maturation: A panel of monoclonal antibodies (CD11a, CD11b, CD15, CD15s, CD18, CD38, CD45, CD65s, CD117, MPO) as described (24) were used to identified the immunophenotyping markers before and after treating AP1060 cells with ATRA. The procedures used for treatment of cells in suspension culture with RA and for the measurement of terminal cell differentiation by the nitroblue tetrazolium dye reduction (NBT) test was performed as previously described (Paietta 1994). Cytologic evaluation of cell differentiation features was determined from Wright –stained cytospin preparations.

Retinoic Acid Binding Assay: Assay the levels of [3H]RA binding in cytoplasmic extracts of AP1060 measured by fast performance liquid chromatography (FPLC) (Jetten 1990). Briefly, 10^8 Cos-1 cells were suspended in 5ml phosphate buffered saline. PSG5 expression vector DNA (400ug), containing either wilde-type long form (P-R-L) or mutant PML-RARa (Pro407Ser), was mixed with the cell suspension for 10 minutes at room temperature. Electro-poration was done by using a Gene Pulser II at 250 uF and 350 V. After 3 days' culture in Dulbecco modification of Eagle medium (DMEM) with 10 % fetal bovine serum (FBS), the cells were harvested for nuclear protein extraction, as described. To test RA binding, 0.2 ml nuclear extract was incubated for 15 hrs at 4 C with 10 nmol/L [^{3}H] RA (30 Ci/mmol) (Dupont-NEN, Boston, MA) in the absence or presence of 200-fold excess of unlabeled RA. The unbound RA was removed by incubation with dextran-coated charcoal for 15 minutes and then centrifuged for 15 minutes at 10000g. The supernatant was analyzed at 4 C by fast performance liquid chromatography (FPLC) , using a Superose 6HR 10/30 size-exclusion column (Pharmacia Biotech, Piscataway, NJ).

RNA and probe preparation: Total RNA was prepared with Trizol (Life Technologies, Inc). For each hybridization, two separate probes were made by using 3DNA Submicro Expression Array Detection Kit (Genisphere, PA): one labeled with Cy3 (control) and another with Cy5 (treated). The 11ul reactions mix was set up by adding 10ug RNA, 3ul Cy3 or Cy5 RT primer (0.067pm0l/ul), and heated at 80°C for 10 minutes to denature the nucleic acid. The following were then added: 1ul Rnase inhibitor (Promaga, MN), 4ul 5X fist-strand buffer (250mM Tris, 375mM KCL, and 15mM MgCl, pH 8.3), 1ul dNTP Mix (10mM each dATP, dCTP, dGTP, and ATTP), 2ul DTT (0.1M), and 1ul Superscript II Reverse Transcriptase (200units/ul; Life Technologies, Inc). The reaction was carried out for 2 hour at 42°C, and stop by adding 3.5ul of 0.5M NaOH/50mM EDTA and heating at 65°C for 10 minutes. After neutralized with 5ul of 1M Tris-HCL(pH7.5), The probes were precipitated by addition of 15ug Linear Acrylaminde and 2.5 volumes of cold ethanol:0.25 volumes 10M Ammonium Acetate, harvested by centrifugation, and resuspended in 15ul Hybridization buffer (40% Formamide/4X SSC/1% SDS).

Hybridization of microarray: Microarray array was printed by Albert Einstein College of Medicine facility, and consisted of the PCR-amplified products of 9256 cloned sequences (Cheung 1999). The slides were prepared for hybridization by moistening over boiling water and then immediately cross-linked with 80 mJ of UV light (UV Stratalinker, Stratagene, La Jolla, CA). Slides were again moisten over boiling water, snap-dried on a hot plate, and soaked for 1 hour in 0.6 M succinic anhydride, 0.02 M sodium borate (pH8.0) in 1-methyl-2-pyrrolidinone. After briefly rinses in 0.1% SDS and distilled water, they were soaked in boiling water for 5 minutes, followed by an ethanol risne at -20°C. The chips were pre-hybridized for 3 hour at 55°C with 2X SSC/0.1%SDS/1%BSA, washed with 2XSSC and 0.2X SSC each for 5 minutes. The hybridization mixture (15ul above probe, 2ul oligo dT blocker and 500ng Cot 1 DNA) was heated at 80°C for 10 minutes. After cooling to room temperature, the mixture was incubated with 5ul of each Cy3 and Cy5 Capture reagent at 55°C for 30 minutes to pre-hybridize the cDNA to the dendrimer, and applied to the pre-hybridized slide,

covered with cover-slip, and hybridized at 55°C for 16 hours. The slide was washed in 2X SSC/0.2% SDS at 60°C for 15 minutes and then in 0.2X SSC/0.1% SDS and 0.1X SSC at 37°C each for 20 minutes.

Scanning and Analysis: Albert Einstein College of Medicine facility provided scanning service. The excitation for Cy3 and Cy5 was at 532 and 633 nm, respectively, and detection centered on 570-580nm and 670-680nm. The emission from the Cy3 and Cy 5 fluorochromes was recorded in two separate high-resolution scans. Two images were superimposed, and the emission at each wavelength was quantified by Genepix 4.0.

The above data were transferred to an Excel spreadsheet, where the signal:background ratio for each channel was calculated as well as the ratio between these ratios (i.e., red signal:background ratio was divided by the green signal:background ratio). The data were then normalized among dots by expressing this value as a ratio to the average of these values for all 9265 genes. The data were transferred to Microsoft Access, where a combination of Access, Microsoft Excel, GeneCluster and TreeView were used for analysis (Eisen 1998).

The time points and RA concentrations were considered as two given agents in a single experiment. For each agent, data for each gene was collected if the signal:background ratio was >1.25 for either the red or green channel.

3. Results

Differentiation response of AP1060 has typical cytological features of the M3 variant form of APL, including a high nuclear:cytoplasmic ratio and cytoplasmic hypogranularity. Following short-term culture in the presence of 100nM ATRA, there was obvious evidence of cellular differentiation compared to untreated cells (Fig. 1A, B). The impression that these nuclear changes reflected increased differentiation was confirmed by NBT test. However, no discernible differentiation was observed with 10nM ATRA (Fig. 1C). After for 5 days, The NBT positive cells increased to 98% in the percentage of 100nM ATRA, compared to increase from the baseline value of 2% in the absence or presence of 10nM ATRA (Fig. 2). At 10nM, expression of myeloid differentiation antigens (CD65s, CD15, CD11b) remained unchanged compared to cultures in the absence of ATRA. However, expression of the precursor antigen CD117 decreased by 50% and CD38 increased from 10 to 44%. At 100nM, all myeloid antigens were present at > 90% of cells while CD117 were lost (Fig. 2).

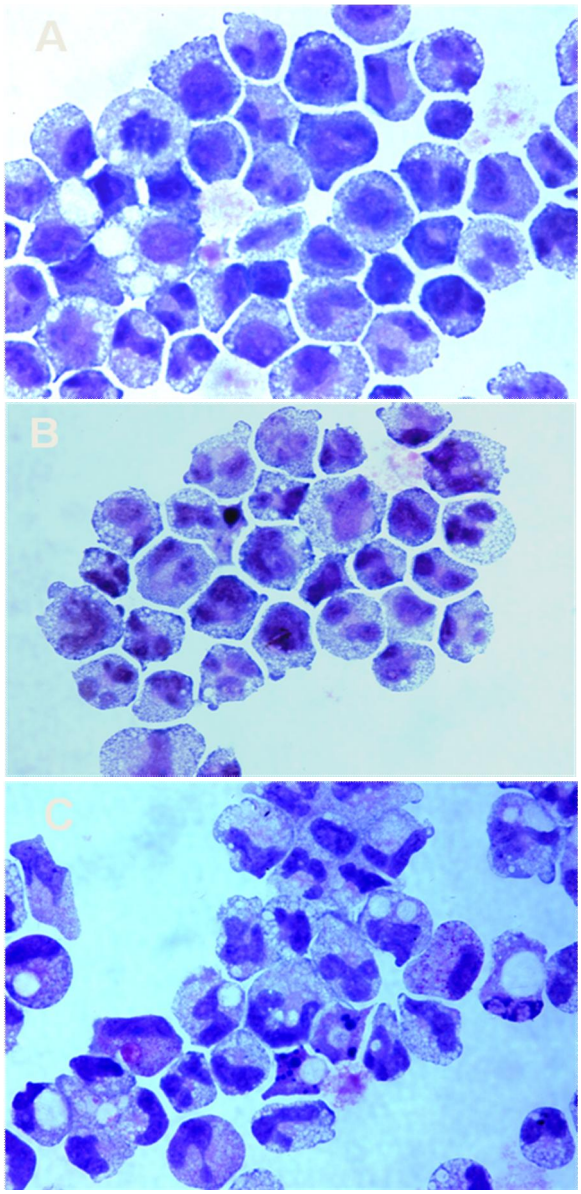

Figure 1. Cytologic evaluation of AP1060 in the absence or presence of ATRA. The cells were cultured for 5 days. Photograhps of cytospin slide were taken at 1000X magnification of modified-wright's stained. (A) without ATRA treatment; (B) 10nM ATRA; (C) 100nM ATRA.

Our published data, which the trans-criptional transaction was performed in COS-1 cells with a reporter vector containing a repeat retinoic acid response element (DR5-tk-luc) to determine whether the RA-dose-response of AP1060 cells dependent on the missense mutation (P407), revealed its abrupt transcriptional transactivation response to ATRA comparing with the L-form PML-RARα (P-R-L) (Sun 2004). This is consistent with the differentiation responsive to ATRA (Fig 2). With

size-exclusion FPLC analysis, RA binding activity was tested by incubating variable concentrations of [3H]RA with nuclear extracts from COS-1 cells which were transiently transfected with pSG5 vector expressing mutant PML-RARα. Nuclear extracts were incubated with the respective concentrations of [3H]RA in the absence or presence of a 200-fold excess of unlabeled ATRA for 15 hr at 4°C. This mutant PML/RARAα completely lack RA binding activity at 1 nM ATRA, but bound ligand in a monomeric and dimeric form respectively at 10 and 100 nM RA. This contrasts to the wild-type L-form (P-R-L) that presents more characteristic high molecular mass, multimeric complexes (Fig. 3). It indicated that the mutant PML-RARα (Pro407Ser) product may represent the mutant PML-RAR homodymers and/or low-molecular-weight complexes with other nuclear proteins at 10 nM ATRA.

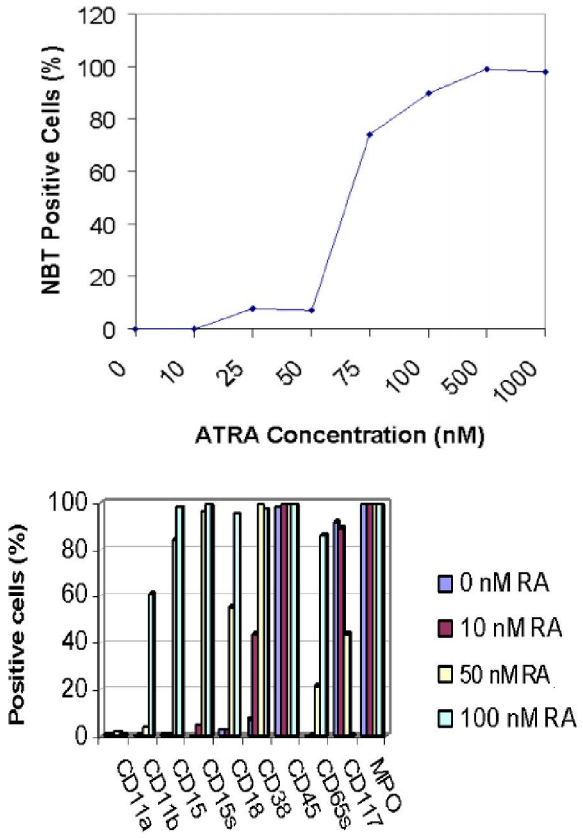

Figure 2. Differentiation response of AP1060 cells to ATRA. The cells were exposed to the indicated ATRA concentrations for 5 days. The percentage of terminally-differentiated cells was measured by NBT and a surface antigen panel.

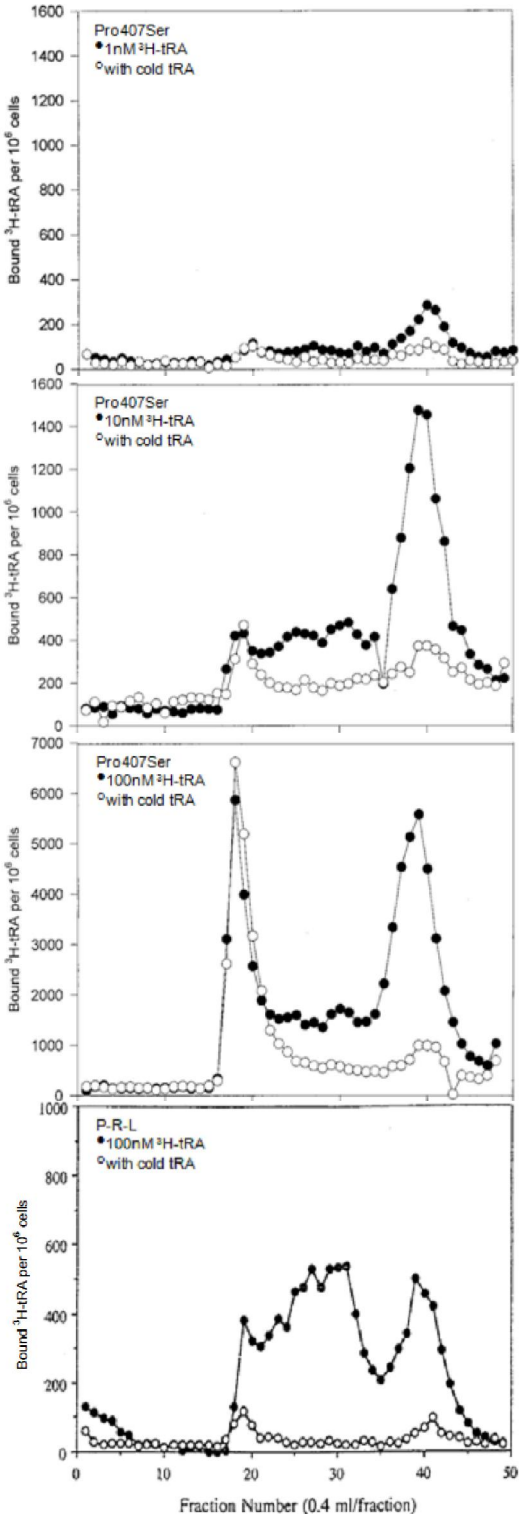

Figure 3. FPLC analysis as described in Methods. (•) [^{3}H]t-RA alone; (o) [^{3}H]t-RA plus 200-fold excess of unlabeled RA. The mutant (Pro407Ser) completely lacked RA binding activity at 1 nM RA, but bound ligand in a monomeric/dimeric form at 10 and 100 nM RA in contrast to the multimeric complexes presented by wild-type L-form (P-R-L).

In separate comparisons of the genes expression with the two different concentrations and time course, the initial ratio of treated to control was set as < 0.8 and > 1.25 (0.8 < t/c > 1.25). 2768 and 3003 genes at a t/c > 1.25 respectively was up-regulated after 12hr and 2623 and 3971 after 24 hr 10nM or 100nM RA exposure.

At a ratio < 0.8, 2330 and 2706 genes were down-regulated at 12 hr and 2001 and 2670 at 24hr. As analyzed at successive 0.25 increments or 0.1 decrements in t/c, the number of up- or down-regulated genes fell off with a bell-shaped distribution, but the numbers were still substantial up to 121 or 536 at 12 hr and 299 or 974 at 24 hr although t/c > 2.5 (Figure 4). Response to ATRA reflected the fact that alterations in expression proceed as a function of time, with increasing time; there was a progressive recruitment of sequences over the 24 h treatment.

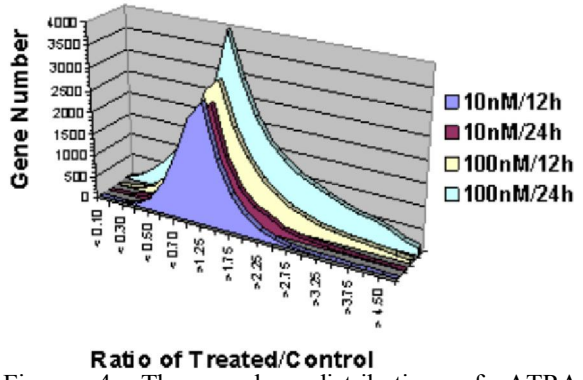

Figure 4. The number distribution of ATRA modulated genes. Screening the genes base on treated/control ratio (t/c) > 1.25 or < 0.8, and on successive 0.25 increments or 0.1 decrements at t/c.

The overall differences in profile of gene expression in 10nM and 100nM, and hence in mechanism of differentiation stimulated by ATRA, were impressive. We selected all genes from the database that were same altered in expression (0.8 < t/c > 1.25) at any time points for 100nM treatment but not for 10nM treatment, and that there were 9 gene cluster in this population of 1550 sequences. 730 among the 1550 sequences present differential expression pattern (Table 1). Other 820 sequences, which present same expression pattern in both 10nM and 100nM, were further dissected according to the criteria that the differential expression genes must show the ratio of the induced to sub-induced (100nM treated/control/10nM treated/control) more than 1.5 or less than 0.6. 240 in upregulated and 120 in downregulated genes set were selected based on these criteria.

In order to further understand the reprogramming of the RA-induced differentiation, we delved more deeply into the differences between the induction and sub-induction by defining gene database into 26 functional gene classes. The class of genes involved in signaling was by far the largest functional class, consisting of 329 members. This class was broadly defined both molecules that may interacted with components of signal transduction pathways (e.g., E-cadherin) and effectors molecules that alter transcription (e.g., fos) as well as signaling components (e.g., kinases) themselves. Within this subclass, 68 genes increased in expression, and 64 were repressed. The 132 genes that encode components of signaling pathways that altered at inducing and sub-inducing concentration, and over the time course of RA treatment. It is interesting that the big differences in genes expression exist between the induced and sub-induced. Thus, extensive alternations in large number of signaling pathways and their components characterize the RA response. The Cluster and TreeView were used to display the profile of the differential expression of these genes (Fig. 5). The results are shown in figure with red representing increased expression, green representing decreased expression, and the magnitude of change depicted by the intensity of color.

Table 1. Profile of the 1550 genes modulated in the induced treatment (100nM) by comparing with same genes set in the subindued (10nM). Upregulaed: Treated/control (t/c) > 1.25; No change: t/c > 0.8 & < 1.25; Downregulated: t/c < 0.8.

100nM / 10nM	Downregulated	No Change	Upregulated
Downregulated	492	77	31
No Change	203		325
Upregulated	8	96	318

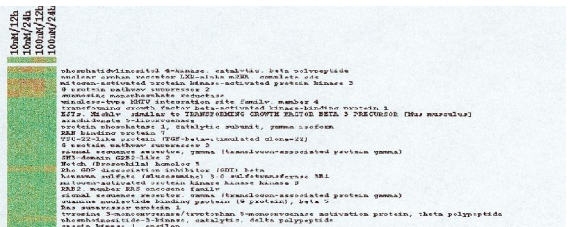

Figure 5. Treeview and Cluster analysis of the alerted expression of signal transduction subset. Red representing increased expression, green representing decreased expression, and the magnitude of change depicted by the intensity of color.

The second functional class of sequences investigated was a subset of 156 genes in the database involved in regulation of cell cycle progression (e.g., cyclins, cyclin-dependent kinases, Rb, myc (Fig. 6). By analysis of the Euclidean distances for this gene subject for the induced and sub-induced, it was first determined that for the functional class of genes responsible for cell cycle progression and arrest, the profile of expression in response to 10nM was less like that initiated by 100nM.

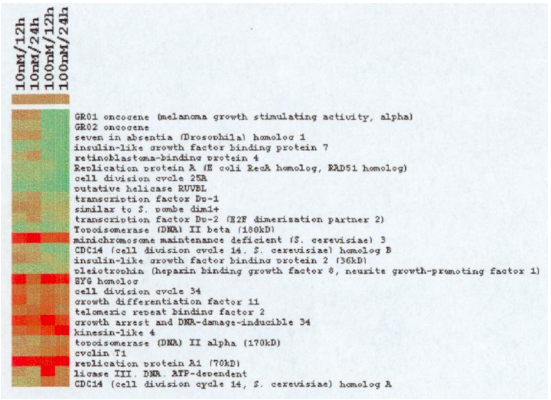

Figure 6. Difference in cell cycle genes set recruited by ATRA. Treeview and Cluster program were used to display sequences that were either induced (red) or suppressed in expression over 48 hours.

Figure 7. Alternation in transcription genes in response to ATRA. The differential expression between the induced and Subinduced cells was analyzed by Treaview and Cluster.

In the third class related to transcription, 20% genes were up-regulated and 33.9% were down-regulated by 100nM RA whereas respectively 11% and 3.6% by 10nM RA over 24 hours (Fig. 7). Most of sequences did not respond to 10nM RA. This illustrates that the transcription s that responded to 10nM and 100nM RA overlapped only to a very limited degree. In the fourth class, cytokines and growth factors, a different profile of genes expression was shown in Fig. 8. In the most interesting gene class, apoptosis, there is no any overlap between 10nM and 100nM RA treatment (Fig. 9). Finally, these data reflect the clear differences in mechanism of comeout treated by 10nM and 100nM RA.

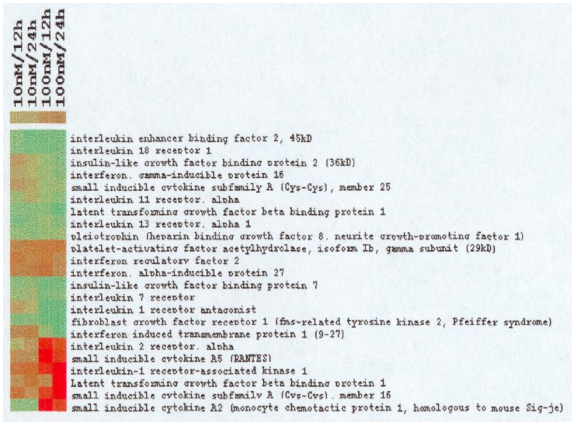

Figure 8. The differential expression in cytokine gene class between 10nM and 100nM ATRA treatment displayed by Treeview and Cluster.

Figure 9. Recruitment of changes in gene expression in response to ATRA. This display all sequences related to apoptosis among 9256 genes printed on chip with Treeview and Cluster.

4. Discussions

One of the least understood aspects of the RA-mediated response of APL cells is the nature of the target genes that are activated leading to differentiation. Isolating the target genes has been an interesting topic. In the past few years, several other groups have tried on NB4 cell line that is inducible with RA and on the specimens of APL patients (Liu 2000; Ki-Hwan 2002; Morikawa 2003; Gutierrez 2005). However, It is impossible to screen the

subsets of genes related to the differentiation although a function assay was used to identify such genes (Khanna-Gupta 2007).

In Ap-1060 cells, a point mutation harbors in RARa portion of PML-RARa that generates an amino change from proline to serine (P407S). It confers AP1060 cell an unique character, abrupt response to ATRA, which was confirmed by either immunophenotype, NBT test or the transcription activity of mutant PML-RARa (P407S)(21). AP1060 cell line allows us to differentially analyze the transcriptional regulation and possible role in APL cells. We have used microarry technology for dissection of the reprogramming, which takes place when RA regulates the pathway of cell cycle arrest, apoptosis balance and terminal differentiation, by comparing induced with sub-induced. The distribution (Fig. 4) of the genes modulated by 10nM or 100nM RA reflected that these genes expression considerably overlapped in induction and sub-induction. In further comparing these genes in table 1, 1550 genes were found to be up- or down-regulated, but only 47.6% genes present a differential expression. These indicate that sub-inducing RA concentration produce substantial changes in gene regulation. But the integration and interaction of the gene modulation by 10nM is not enough to initiate reprogramming cell's differentiation.

This reprogramming is implicated the complicated procedure how pathways of cell cycling and cell death are coordinated to maintain differentiation. In four functional classes we have presented, genes involved in signal transduction, cell cycling, and apoptosis, there are a lot of significant modulations in expression of many genes that are generally considered fundamentally important in pathways of cell maturation, and many of these distinguish the induced-response from sub-induced response. Thus, the integration and interaction of modulation of large numbers of genes reflect the regulatory and functional circuits that determine the probability of a cell's behavior, such as continuation of proliferation, or cell cycle arrest accompanied by differentiation and/or apoptosis. Table 2 reflects such kind of modulation.

Many studies suggested that the initiation of differentiation required the transcriptional activation of specific genes leading to proliferation arrest and cell cycle exit (Rousselot 1999). Supporting this notion, most of G-protein family (RAB 13, Rho, and Rap1) were up-regulated whereas RAB interaction factor and G protein pathway suppressor were down-regulated. Along these same lines, genes favoring G1-S/G2-M transition, such as Mitogen-activited protein kinases (MAPK) and Transforming growth factor were repressed during inducing RA treatment. The fact that most of these modulations take place only at 100 nM RA treatment suggests that the sub-induced situation results from the modulations at 10nM that can not drive cells out off cell cycle (Table 2).

Table 2. The genes modulated by ARTA display the reprogramming map of cell differentiation.

			10nM RA		100nM RA	
			12h	24h	12h	24h
proliferation arrest and cell cycle exit	G protein family	RAB13 ↑	0.9	0.41	1.67	2.4
		RAB 36 ↑	0.88	0.87	1.26	1.7
		RAB interaction factor ↓	1.3	1.3	1.2	0.69
	G1-S/G2-M transition	M-phase phosphoprotein 1 ↑	1.1	1.3	1.3	2.69
		Growth arrest-specific 2 ↑	1.0	0.9	0.84	1.77
Cross-talking	G proteins – MAPK	in cooperative or antagonistic manner				
	TNF-INF – IL	TNF 11 ↑	0.87	0.95	1.1	2.2
		Interferon 2-inducible protein ↑	1.1	1.3	5.95	7.1
		IL 1 receptors ↑	0.61	1.0	0.8	1.8
		IL receptor 1 angonistor ↓	1.1	0.9	0.13	0.2
	cAMP related pathway	cAMP-dependent protein kinase	1.1	0.56	1.8	1.9
		cAMP responsive element modulator	0.7	0.55	0.54	1.44
Apoptosis and survival		BCL2-related protein A2 ↑	1.1	0.83	6.1	13.4
		Seven in absentia homologies (SIAH)↑↓	1.1	1.1	0.48	0.31
Differentiation		CEBPB ↑	1.6	1.9	8.7	5.7
		STAT3 ↑	1.97	1.18	7.1	13.4
		GATA Binding protein 2 ↓	2.1	1.3	0.72	0.70

A cross-talk between G-proteins and/or MAPK regulates various cellular functions in a cooperative or antagonistic manner (Matozaki and Sebolt-Leopold 2000; Pruitt 2001). Another cross-talk between Tumor necrosis factor and Interleukins may exist in RA-induced differentiation, in which TNF 1, 11 and IL receptors (e.g., IL-1R, IL-2Rα) were up-regulated and TNF receptor associated factor 3 (TRAF), TRAF interacting protein whereas IL receptor 1 angonistor, IL 8, IL6 were down-regulated. The role of TNF-related apoptosis-inducing ligand (TRAIL) has been confirmed in RA-induced apoptosis in APL (Altucci 2001). TRAF proteins are involved in signaling by members of the IL-1R/Toll receptor family or IL-15R and IL17R (Wajant 2001). TRAIL activation was a late event during RA-inducing differentiation ((Altucci 2001). Therefore, the early expression of IL1 may mediate the pathway related to TRAIL. Remarkably, TRAF proteins have also been shown to be involved in a functional MAPK cascade. Recently, FOXO3A, a target of TRAIL, was regarded as a key molecule for ATRA-induced granulocytic differentiation and apoptosis in APL(Yasuhiko 2010).

It is interesting detecting the increased expression of cAMP related protein such as cAMP phosphodiesterase, cAMP-dependent protein kinase and cAMP responsive element modulator. The sythestic treatment of CPT-cAMP and RA drives RA resistent NB4 subline, which contains a mutation (P407L) in PML-RARa, differentiation. Activation of cAMP-protein kinase pathway by cAMP correlated with the induction of differentiation and apoptosis (Kitamura 1997; Duprez 1996; Srivastava 2000).

Apoptosis antagonist, BCL2-related protein A2 was sharply up-regulated. Seven in absentia (Drosphila) homologies (SIAH-1, 2, and 3), in which SIAH-1, a p53-p21Waf-1 inducible gene, can induce apoptosis and promote tumor suppression (Roperch 1999), were repressed. These reflect the balance between apoptosis and differentiation to maintain the cell survival and maturation process.

The transcription factor C/EBP (CCAAT /enhancer binding protein) is a target gene of PML/RARα, and is crucial for induced myeloid differentiation in APL (Duprez and Truong 2003**;** Rego 2009). Here we found both of CEBPδ and GATA2 present opposite expression, up-regulated and down-regulated, separately in the induced and sub-induced RA treatment. CEBPβ presented 3-5 fold increasing modulation. Of note, GATA2 appears critically involved the survival and growth of multi-potential progenitors.[46] It was reported that the promyelocytic leukemia protein (PML) could complex with GATA2 and potentiated its transactivation capacity (Tsuzuki 2000).

The different profiles of gene expression induced by the inducing and sub-inducing RA concentration exhibited the differing kinetics of transcription activation. Our results indicated that the recombinant mutant PML/RARα bound [^{3}H] RA to saturation at 10nM, but presents monomeric comparing to dimeric form at 100nM, and in a transfection reporter vector assay using a direct-repeat RA response element/promoter, the transcriptional activation RA dose-response curve precisely followed the AP-1060 cell differentiation (Sun 2004). It imply that the transcription was activated by the protein interactions initiated by 100nM RA. Furthermore, Retinoid X receptors (RXR) that interact with RAR or PML/RARα to form functional hetrodimer shown differential expression in which RXRα was up-regulated by sub-inducing RA concentration and RXRγ presented the decreasing pattern in inducing RA treatment. Thus, the alerted population of nuclei receptors or transcription factors, which associate or dimerize with RARα or PML/RARα, may affect their target transcription. Therefore, there could be a same competition mechanism for them to bind to CoA or CoR molecular (Perez 1993). The dissociation of corepressors from this mutant PML/RARα was not found at both of inducing and sub-inducing RA treatment (Sun 2004). It may be the possible explanation that the different dimers or multimeric complexes between RARα or PML/RARα and other transcription factors posse the different affinity with tRA so that their interactions were disrupted by different kinetics.

In summary, we have established a new cell system, AP-1060, for RA-induced differentiation research, in which the mutation (P407S) in RARa portion of PML-RARa coffers this cells the abrupt response to ATRA. On the base of this cells, the large-scale profiling of gene expression was performed to characterize the response of APL cell to RA. The 730 genes were identified in differential expression between induced cells and sub-induced cells. It suggests that the AP1060I cell line provides a system for dissecting genes with different regulatory mechanisms and for identifying key differentiation-controlling genes, and that the pathway involved in RA-response may be the target for treating the patients who resistant to RA.

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