Ribavirin Acts *via* Multiple Pathways in Inhibition of Leukemic Cell Proliferation

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Abstract. *The aim of this study was to elucidate the anticancer activity of Ribavirin, an antiviral drug and a known inhibitor of inositide-5'-monophosphate dehydrogenase. Materials and Methods: Using human cancer cell lines, the potential of the drug to inhibit growth and induce the apoptotic and differentiation pathways was investigated by cytological methods. The effect exerted upon gene expression was studied in K562 cells by Q-PCR. Results: Treatment with Ribavirin resulted in a significant growth inhibition (IC_{50}=15 μM) via activating apoptosis and the differentiation pathway in K562 cells. It also modulated the expression of about 60 out of 85 genes having roles in cell proliferation, purine biosynthesis, translation initiation, oncogenic signaling and cell survival ($p<0.05$). Conclusion: Ribavirin is a potent anticancer agent, being a strong inducer of apoptosis and a moderate inducer of differentiation in K562 cells. These effects are mediated through the modulation of key molecular and metabolic pathways.*

Ribavirin (1-β-D-ribofuranosyl-1,2,4,-triazole-3-carboxamide) was developed as an anti-viral agent against several DNA and RNA viruses (1). The mechanisms responsible for its antiviral activity are well-understood.

Soon after the discovery of the broad-spectrum antiviral activity of Ribavirin, the drug was proposed as a specific inhibitor of inositide-5'-monophosphate dehydrogenase (IMPDH), a cellular enzyme catalyzing the rate limiting step of *de novo* GTP synthesis (1, 2). Ribavirin blocks the enzyme at the IMP-XMP binding site (3). Weber and co-workers showed that IMPDH expression and activity are markedly elevated in various animal and human tumor types and are closely linked to transformation and proliferation of malignant cells (4-6). Therefore, IMPDH inhibitors were suggested as strong candidates for anticancer chemotherapy (4). IMPDH I is constitutively expressed and is the predominant form in normal cells, while IMPDH II is selectively up-regulated in neoplastic and proliferating cells, including leukemic cells (7-9). The increased activity of IMPDH in cancer cells results in an elevated capacity to provide GDP and GTP. Guanine nucleotides are required for several metabolic and signaling pathways and functions of cells (4, 10, 11). Previous results with tiazofurin provided the first evidence that the proliferative arrest and differentiation of BCR-ABL positive human leukemia cells, induced by inhibition of IMPDH, are mediated by the profound down-regulation of RAS and MYC oncogenes (12-14). Induction of differentiation by tiazofurin was also observed *in vivo* in patients with refractory acute myelogenous leukemia (15-18). Whereas the biochemical programs induced by the clinically used IMPDH inhibitors are well described, the effects of Ribavirin on gene expression in leukemia cells are poorly understood.

Taylor and co-workers provided evidence that treatment with Ribavirin has very little, if any, effect on gene expression on normal blood (PBMC) cells (19). Kentsis and colleagues showed that Ribavirin treatment can act through the inhibition of translation but has no effect on either the chromatin structure or the transcription of some oncogenes such as cyclin D1 and VEGF (20, 21). Apart from these preliminary results, the molecular targets and pathways affected by IMPDH inhibitors are poorly understood.

This paper evaluates the molecular mechanism of action of Ribavirin as a single drug in cancer cells using both traditional cytological methods and pathway-specific TaqMan Low Density Array (LDA) analysis. The purpose of this investigation was to provide evidence on the anticancer effects of Ribavirin and to gain a deeper insight into the

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molecular mechanisms of its antiproliferative action. The results are novel and confirm the role of specific pathways in the effect of Ribavirin treatment on leukemic cells.

Materials and Methods

Cell lines and tissue culture. MCF-7 breast cancer cells and HepG2 human hepatocellular carcinoma cells were grown in 10% DMEM medium (Sigma Chemical Co., St. Louis, MI, USA) supplemented with 10% foetal bovine serum (GIBCO, Germany). OVCAR-5 ovarian cancer cells and K562 BCR-ABL positive human leukemia cells were grown in suspension culture in RPMI-1640 medium (Sigma) supplemented with 10% foetal bovine serum. Cells were maintained in culture at concentrations between 1×10^5 and 1×10^6 cells/mL by sub-culturing every 2-3 days. For individual gene expression experiments, cells in log phase growth were cultured at initial seeding concentrations of 3×10^6 cells/mL with or without addition of Ribavirin.

Growth inhibition assay. Growth inhibition assay was carried out as previously described (22). Briefly, 5×10^4 log phase cells/mL were seeded in triplicates in culture flasks (Greiner, Frickenhausen, Germany). Next day, cells were treated with 0, 1, 10, 20, 50, 100 and 150 μM Ribavirin (Sigma). For measurement of toxicity, cell viability was monitored daily by trypan-blue dye exclusion test at each concentration up to five days. The percentage growth inhibition of treated cultures as compared to untreated cultures was plotted in growth inhibition curves for each concentration and duration. The number of surviving cells was used in a regression curve analysis to determine the IC_{50} values at different timepoints.

Clonogenic assay. The standard agar colony assay for K562 leukemia cells was carried out as previously described (12). Briefly, 1000 cells per 5 mL of RPMI 1640 medium were plated in 50 mm Petri dishes (Greiner). The medium was supplemented with 20% fetal calf serum (GIBCO), penicillin (100 units/mL), streptomycin (50 mg/mL), a small volume of drug solution to achieve final concentrations of 10, 30 and 50 μM, respectively, and 0.33% agar (Noble-agar; Difco Laboratories, USA). Fourteen days later the colonies (containing more than 50 cells) were scored under an inverted microscope. Untreated control plates yielded about 800 colonies per plate. The percentage survival of treated cultures as compared to untreated cultures was plotted in survival curves. IC_{50} value was determined using regression curve analysis.

Morphological signs and assessment of apoptosis. Cells at a 5×10^4/mL starting density were treated with 15 or 50 μM of Ribavirin for 6, 24 and 48 hours. At the end of each incubation period, cells were rinsed with 1×PBS, centrifuged and stained with hematoxylin-eosin on microscopic slides. One thousand cells per sample were analyzed using light microscopy for appearance of morphological signs of apoptosis (23).

Cellular differentiation. K562 leukemia cells were treated with 15, 50 or 145 μM of Ribavirin up to 120 hours. Benzidine staining was applied for detection of erythroid differentiation as described previously (12). To measure the capacity of the cells to undergo erythroid differentiation, the percentage of benzidine positive cells were calculated. One thousand cells were counted by the use of light microscopy.

Total RNA isolation and cDNA synthesis. High-purity RNA was extracted from cells treated with Ribavirin for 0.5, 1, 3, 6, 9 and 12 hours (15 μM) or 3, 12 and 48 hours (50 μM) using RNAqueous kit (Applied Biosystems, Foster City, CA, USA). Three parallels of RNA of equal concentration were pooled and reverse transcribed using 250 ng of total RNA and 125 U Multiscribe Reverse Transcriptase according to the manufacturer's protocol (High-Capacity cDNA Reverse Transcription Kit, Applied Biosystems).

Real-time (TaqMan) PCR assay. Validated TaqMan PCR assays for 9 previously selected genes and the major BCR-ABL transcript were performed on K562 cells treated with 15 μM Ribavirin for 12 hours on an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems) according to the manufacturer's instructions. The target gene expression was normalized to the geometric mean of β2 microglobulin and RPLP0 as internal controls and the fold-changes were determined by the $2^{-\Delta\Delta Ct}$ method (24). All reactions were done in quadruplicate. The Sequence Detection Software SDS 2.2 (Applied Biosystems) was used for data analysis.

TaqMan low density array. Gene expression was measured at three timepoints (3, 12 and 48 h) in both Ribavirin treated (50 μM) and untreated cells. Arrays with four sets of 96 genes were used. Each reaction was carried out using 0.5-1 ng mRNA as template. Primer/probe sets were chosen from the validated collection of TaqMan Gene Expression Assays (Applied Biosystems). Key genes located in important cancer-related pathways were selected as targets from the Panther database (www.pantherdb.com) (25). All procedures were carried out as recommended by the manufacturer. The QRT-PCR runs were performed on an Applied Biosystems 7900HT instrument. For data normalization the geometric mean of two control genes (RPLP0, β2-microglobulin) was applied. The normalized gene expression was compared to that of untreated cells as calibrators at each time point using the $2^{-\Delta\Delta CT}$ method. All data analysis was performed using the StatMiner software (Integromics). For visualization of results, JColorGrid software was applied (26). Nine genes were excluded from further evaluation as a result of their obvious amplification failure. Time-independent responses were calculated from the area under the curves drawn on the basis of the three measured timepoints using ImageJ software (27). Only genes with an expression fold-change more than 1.2 were included in further evaluation.

Results

Antiproliferative action of Ribavirin. A single treatment with Ribavirin resulted in a significant growth inhibition of ovarian cancer (OVCAR-5), leukemia (K562), breast cancer (MCF-7) and hepatocellular carcinoma cells (HepG2) in a dose- and time-dependent manner (Figure 1A). Growth curves measured over 5 days with 0, 1, 10, 20, 50, 100 and 150 μM Ribavirin yielded IC_{50}=15 μM for K562, 45 μM for HepG2 cells, 35 μM for MCF-7 and 10 μM for OVCAR-5 cells at 120 hours. The survival curve measured over 14 days in agar clonogenic assay yielded IC_{50}=15 μM (Figure 1B). Ribavirin concentrations equal to or above 15 μM were used for further treatment.

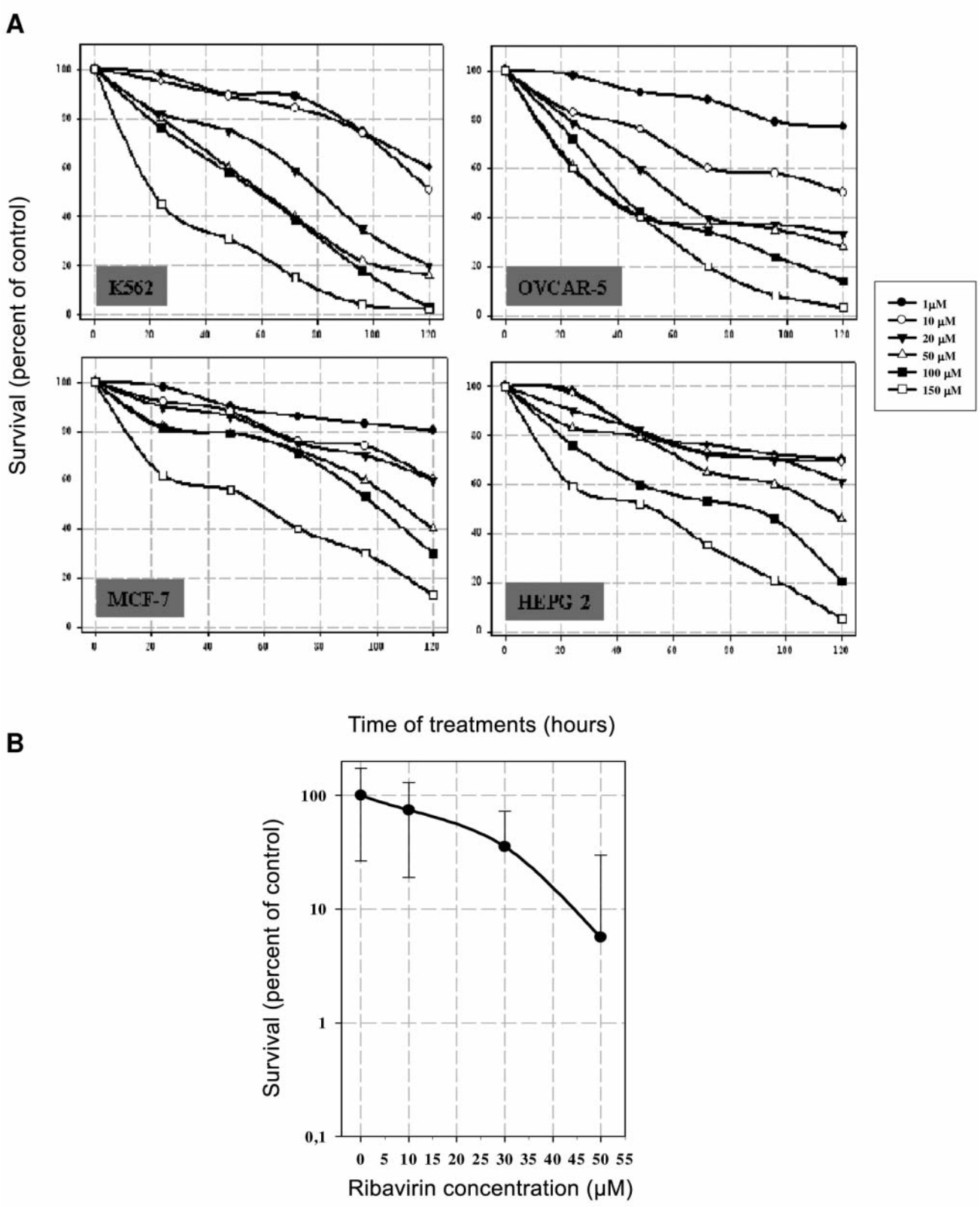

Figure 1. *Ribavirin-induced antiproliferative action in various tumor cells lines. Ribavirin induces cytotoxicity as demonstrated by survival curves on K562, OVCAR-5, MCF-7 and HepG2 human tumour cells (Panel A, SE values were <±10%) and soft agar clonogenic assay for K562 cells (Panel B).*

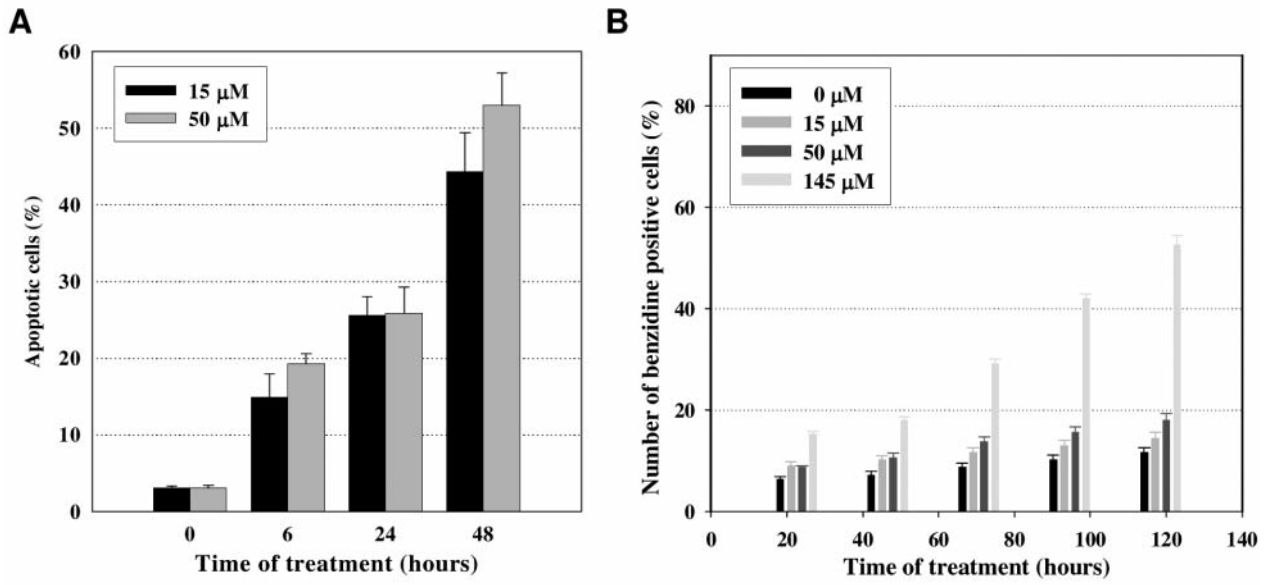

Figure 2. *Induction of apoptosis and erythroid differentiation by Ribavirin in K562 leukemia cells. Leukemia cells were treated with various concentrations of Ribavirin for different time periods as indicated. The number of apoptotic cells is shown as percentage of the total number of cells (Panel A). The number of differentiated cells is indicated as percentage of the number of surviving cells (Panel B). The results are means of triplicates.*

Induction of apoptosis and erythroid differentiation. Ribavirin proved to be a potent inducer of apoptosis in K562 human leukemia cells. The proportion of cells showing morphological features typical of apoptosis (shrinkage of cytoplasm, apoptotic bodies) increased in a dose- and time-dependent manner in K562 cell line (Figure 2A). The ability of Ribavirin to induce erythroid differentiation was evaluated in K562 cells treated with Ribavirin for up to 5 days. Using benzidine which stains globin specifically, erythroid differentiation could be detected (see Materials and Methods). Treatment with 145 μM Ribavirin induced a 5-fold increase in the number of cells showing signs of differentiation (Figure 2B). Lower drug concentrations (15 or 50 μM) were not effective.

Real-time quantitative PCR and Taqman LDA analysis of Ribavirin treatment in K562 cells. Based on previous results, the 15 μM (IC_{50}=120 h) concentration was chosen to explore the potential expression changes exhibited by 10 genes as early response to the treatment (Figure 3) (14). Furthermore, 96 genes were selected with relevance to the major pathways that were expected to be responsible for the effects of Ribavirin on apoptosis, differentiation, translation initiation and guanylate metabolism. This selected group also included oncogenes, tumor suppressors and other factors belonging to main cancer-related signaling pathways (50 μM, up to 48 h) (Figure 4).

A 3-fold up-regulation of the BAX/BCL-2 mRNA ratio was measured in an interval between 30 min and 3 hours using 15 μM Ribavirin (Figure 3). In response to treatment with 50 μM of drug, pro-apoptotic factors showed either down-regulation (BAX, BID, BIRC5, FAD, TNFRSF1A, FAS) or up-regulation (BAD, BBC3, PMAPI) of gene expression at all tested time points up to 48 hours. Several genes belonging to the same group were down-regulated at 3 hours, although they showed up-regulation at 48 hours (Caspase genes, NFKB2 and NFKBIA) (Figure 4).

The majority of the tested differentiation genes (HBB, Globin A, MPO) showed increased expression upon Ribavirin treatment independently of the drug concentration, whereas AKT1 was down-regulated until 48 hours using 50 μM Ribavirin (Figures 3, 4).

Most genes of the purine metabolism and translation initiation were down-regulated (IMPDH II, PARP1, EIF2A, EIF2B1 and EIF4G1). These changes could be experienced as early as 3 hours after drug administration and were maintained up to 48 hours. In contrast, although early down-regulation of GMPS, GUSB, HPRT1 and EIF4E genes was detected, they were found up-regulated at later time points (Figure 3, 4).

The mRNA expression of the selected oncogenes (ABL, CCND1, CCNE1, CDKN1B, HRAS, MDM2, MYC, PAK1, TERT) were unanimously down-regulated whereas the tumor suppressors RB1, TP53 and TSC2 showed elevated mRNA levels up to 48 hours using 50 μM of the drug (Figure 4). Treatment with 15 μM Ribavirin also resulted in a significant decrease of C-MYC, TERT, b3a2 transcript and ABL expression while the level of CIP1/WAF1 was increased for 12 hours (Figure 3).

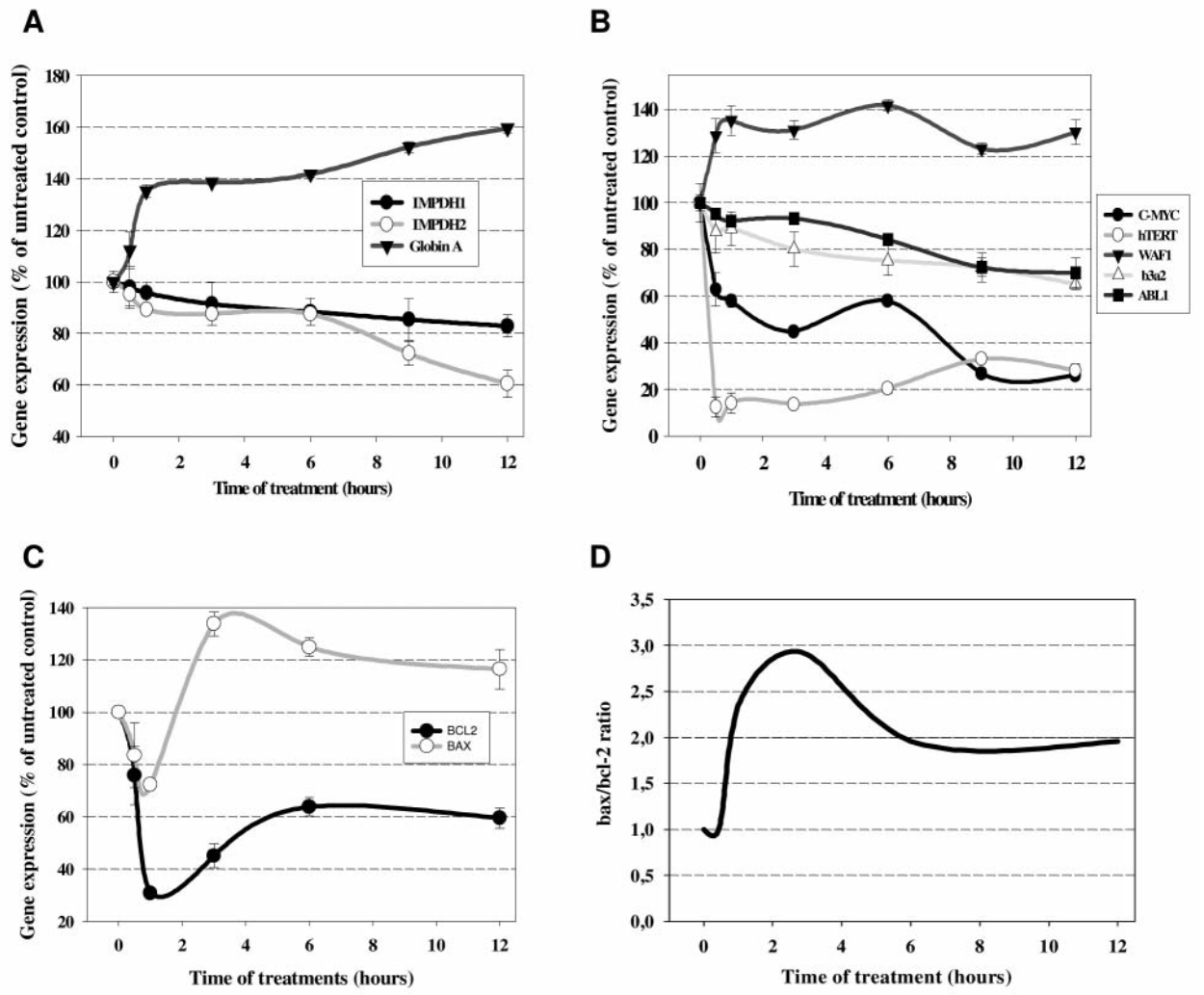

Figure 3. *Expression profile of 10 genes affected by Ribavirin treatment. Q-PCR results after 15 μM Ribavirin treatment are presented (panels A, B and C). Means of three experiments were calculated; SE values are indicated. The expression of target genes was normalized to the geometric mean of RPLP0 and β2 microglobulin expression. Untreated cells were applied as calibrator. Panel D demonstrates the early increase in the BAX/BCL2 mRNA ratio calculated from data shown in Panel C.*

Several members of the group containing genes with a role in further signal transduction pathways were down-regulated during the entire tested interval upon treatment with 50 μM Ribavirin (CTNNB1, IFNAR1/2, PRKAR1A, PRKCA). In contrast, HIF1A1, HSPB2, TPPP and VEGF genes showed increased expression at each tested time point. After an early (3 h) decrease in expression, IGF1, IL1R1, JAK1, PXN, SOS1 and STAT1 genes showed late up-regulation as could be detected at 48 hours (Figure 4).

The time-independent net alteration in the expression of the majority of the tested genes was also calculated and summarized in Table I (50 μM). Taken together, these results indicate that Ribavirin acts on multiple levels in inducing the apoptotic and differentiation programs as well as modifying several other cancer-related processes (Figure 5).

Discussion

The results of the cellular and molecular analyses demonstrate that a complex series of molecular events prepares Ribavirin-treated cells for the inhibition of proliferation, the induction of programmed cell death and/or initiation of differentiation. This study presents the first quantitative screen for changes in expression of 96 cancer-related genes after Ribavirin exposure. The data support the model that Ribavirin has antiproliferative and cytotoxic effects on breast cancer (MCF-7), ovarian cancer (OVCAR-5), hepatocellular carcinoma (HepG2) and leukemia (K562) cells in a time- and dose-dependent manner.

Here it is reported that Ribavirin treatment of K562 cells resulted in a major decrease of the IMPDH II mRNA level,

Table I. *Expression of genes grouped according to biological role. The time-independent net fold-changes were calculated as described in Materials and Methods section.*

Gene Name	Assay #	Sequence description	Fold-change	
			UP	DOWN
APOPTOSIS				
Extrinsic pathway				
FAS	Hs00163653_m1	Fas (TNF receptor superfamily, member 6)		–1.81
FADD	Hs00538709_m1	Fas (TNFRSF6)-associated via death domain		–1.44
TNFRSF1A	Hs01042313_m1	tumor necrosis factor receptor superfamily, member 1A		–1.56
BID	Hs00609632_m1	BH3 interacting domain death agonist		–1.59
TRADD	Hs00182558_m1	TNFRSF1A-associated via death domain	1.25	
CRADD/RIP	Hs00187009_m1	CASP2 and RIPK1 domain containing adaptor with death domain	1.28	
NFKB1	Hs00765730_m1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)		–1.37
NFKB2	Hs00174517_m1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	1.26	
NFKBIA	Hs00153283_m1	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha		1.25
CASP10	Hs00154268_m1	caspase 10	2.12	
Intrinsic pathway				
BBC3/PUMA	Hs00248075_m1	BCL2 binding component 3	1.51	
PMAIP1/NOXA	Hs00560402_m1	phorbol-12-myristate-13-acetate-induced protein 1	1.43	
CASP2	Hs00154242_m1	caspase 2	1.42	
CASP9	Hs00154260_m1	caspase 9	1.32	
Common pathway				
CASP3	Hs00234387_m1	caspase 3	1.39	
CASP6	Hs00154250_m1	caspase 6	1.55	
CASP7	Hs00169152_m1	caspase 7	1.88	
DIFFERENTIATION				
AKT1	Hs00178289_m1	v-akt murine thymoma viral oncogene homolog 1		–1.40
CD3E	Hs01062241_m1	CD3e molecule, epsilon (CD3-TCR complex)	1.45	
CD79A	Hs00233566_m1	CD79a molecule, immunoglobulin-associated alpha	1.30	
HBB	Hs00758889_s1	hemoglobin, beta	2.37	
MPO	Hs00165162_m1	myeloperoxidase	2.66	
ONCOGENES/TUMOR SUPPRESSORS				
ABL1	Hs00245445_m1	v-abl Abelson murine leukemia viral oncogene homolog 1		–1.92
CCND1	Hs00277039_m1	cyclin D1		–1.75
CCNE1	Hs00233356_m1	cyclin E1		–2.01
CDKN1A	Hs00355782_m1	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.49	
CHEK1	Hs00176236_m1	CHK1 checkpoint homolog (S. pombe)		–1.55
HRAS	Hs00610483_m	v-Ha-ras Harvey rat sarcoma viral oncogene homolog		–1.66
MDM2	Hs01066938_m1	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein		–1.38
MYC	Hs00153408_m1	v-myc myelocytomatosis viral oncogene homolog		–2.04
PAK1	Hs00176815_m1	p21/Cdc42/Rac1-activated kinase 1		–1.43
RB1	Hs00153108_m1	retinoblastoma 1	1.63	
RHOA	Hs00357608_m1	ras homolog gene family, member A	1.29	
TERT	Hs00162669_m1	telomerase reverse transcriptase		–2.49
TP53	Hs00153349_m1	tumor protein p53 (Li-Fraumeni syndrome)	1.65	
TSC1	Hs00184423_m1	tuberous sclerosis 1	2.115	
TSC2	Hs00241068_m1	tuberous sclerosis 2	1.37	
SIGNALING				
ATM	Hs00175892_m1	ataxia telangiectasia mutated	1.34	
CREB1	Hs00231713_m1	cAMP responsive element binding protein 1	1.32	
CTNNB1	Hs00170025_m1	catenin (cadherin-associated protein), beta		–1.60
HIF1A	Hs00936368_m1	hypoxia-inducible factor 1	1.40	
HSPB2	Hs00155436_m1	heat shock 27kDa protein 2	1.73	
IFNAR1	Hs00265057_m1	interferon receptor 1		–1.24
IL1R1	Hs00991002_m1	interleukin 1 receptor	1.38	
JAK1	Hs00233820_m1	Janus kinase 1	1.35	
MAPK8	Hs00177083_m1	mitogen-activated protein kinase 8	1.35	
PRKCA	Hs00176973_m1	protein kinase C, alpha		–1.23
PXN	Hs00236064_m1	paxillin	1.28	

Table I. *continued*

Table I. *continued*

Gene Name	Assay #	Sequence description	Fold-change	
			UP	DOWN
STAT1	Hs00234829_m1	signal transducer and activator of transcription	1.25	
TPPP/GSK3	Hs00389316_m1	brain-specific protein p25 alpha	2.29	
VEGF	Hs00900054_m1	vascular endothelial growth factor	1.45	
TRANSLATION INITIATION				
EIF2A	Hs00230684_m1	eukaryotic translation initiation factor 2A		–1.32
EIF2B1	Hs00426752_m1	eukaryotic translation initiation factor 2B		–1.48
EIF4G1	Hs00191933_m1	eukaryotic translation initiation factor 4 gamma, 1		–1.26
GUANYLATE METABOLISM				
GMPS	Hs00269500_m1	guanine monphosphate synthetase	1.34	
HPRT1	Hs99999909_m1	hypoxanthine phosphoribosyltransferase 1	1.32	
IMPDH1	Hs00265302_m1	IMP (inosine monophosphate) dehydrogenase 1		–1.28
IMPDH2	Hs00168418_m	IMP (inosine monophosphate) dehydrogenase 2		–1.68
PARP1	Hs00242302_m1	poly (ADP-ribose) polymerase family, member 1		–1.41

which is the predominantly expressed IMPDH isoform in cancer cells (7, 8). The increased expression of GMPS may occur as a compensation for the inhibition of IMPDH, the key enzyme of purine metabolism.

In this study, Ribavirin induced programmed cell death in K562 cells in a dose- and time-dependent manner. A significant (almost 50%) increase in the percentage of cells undergoing apoptosis was observed at 48 hours after treatment with 50 μM of Ribavirin, which is in line with a report by Schlosser and coworkers (28). They showed that combined treatment with Ribavirin and interferon-α can sensitize HepG2 cells for apoptosis, which correlated with caspase-3 activation and stimulation of the death receptor CD95/FAS. Surprisingly, down-regulation of genes belonging to the CD95/FAS pathway (FAS, FADD) was observed, but we found elevated expression of those mediating the effects of TNFR superfamily members (TNFSR1A, TRADD, RIP). Up-regulation of several genes of the intrinsic pro-apoptotic pathway (PUMA, NOXA, TP53, CASP2, CASP9) as well as CASP3, CASP6 and CASP7 that belong to the effector arm of the apoptotic machinery was also detected. These results showing that Ribavirin can activate both the extrinsic and intrinsic apoptotic pathways are in line with the elevated BAX/BCL-2 ratio observed during the first 3 hours of treatment with 15 μM Ribavirin and strongly support the cytological findings.

A high dose of Ribavirin caused an elevation in the number of differentiated cells, which steadily increased with the duration of drug exposure in K562 cells. The results seem to support the previous observations that Ribavirin can induce moderate differentiation compared to other inhibitors of the enzyme (12, 14, 29, 30). At the same time, Ribavirin treatment resulted in a significant up-regulation of HBB, Globin A and MPO genes but decreased the expression of MYC and AKT1, a well-known survival gene. Early alterations in gene expression profiles are in line with the cytological signs observed at later time points (96 and 120 h). These molecular changes could be responsible for inducing both the differentiation and the apoptotic processes (13, 22, 31, 32).

Evidence has been provided that treatment with Ribavirin (50 μM) had strong inhibitory effects on the expression of ABL1, MYC, Ha-RAS, hTERT, CCND1, CCNE1, CHEK1, PAK1 and MDM2 genes up to 48 hours (Figure 4). Using 15 μM of the drug, similar down-regulation was detected in the case of MYC, TERT and ABL1 at early stages of the treatment (Figure 3). Meanwhile, classical tumor suppressors such as TP53, RB1 and TSC were up-regulated. According to an earlier report, exposure of K562 cells to tiazofurin, another potent inhibitor of IMPDH, resulted in significant down-regulation of the expression of MYC and Ha-RAS, which is in agreement with the results of the present study (12).

Ribavirin also caused a significant decrease in the expression of the major BCR-ABL mRNA variant (b3a2), which may open up new opportunities towards a specific drug combination for the therapy of CML. There is an increasing body of evidence that targeted inactivation of oncogene products may counteract the effects of genetic events underlying tumorigenesis and may serve as an effective therapy against cancer. These results may be even more noteworthy in the light of the fact that "rehabilitation of cancer" could be done with MYC inactivation alone (33).

A decrease in the expression of eIF4G, eIF2A and eIF2B genes could be observed after treatment with 50 μM of Ribavirin in K562 cells. These results strongly support the inhibitory role of Ribavirin on translation initiation. A further translation inhibitory mechanism of the drug was reported by Kentsis and co-workers, which involves binding of eIF4E at the functional site used by the 7-methyl guanosine mRNA cap (20, 21).

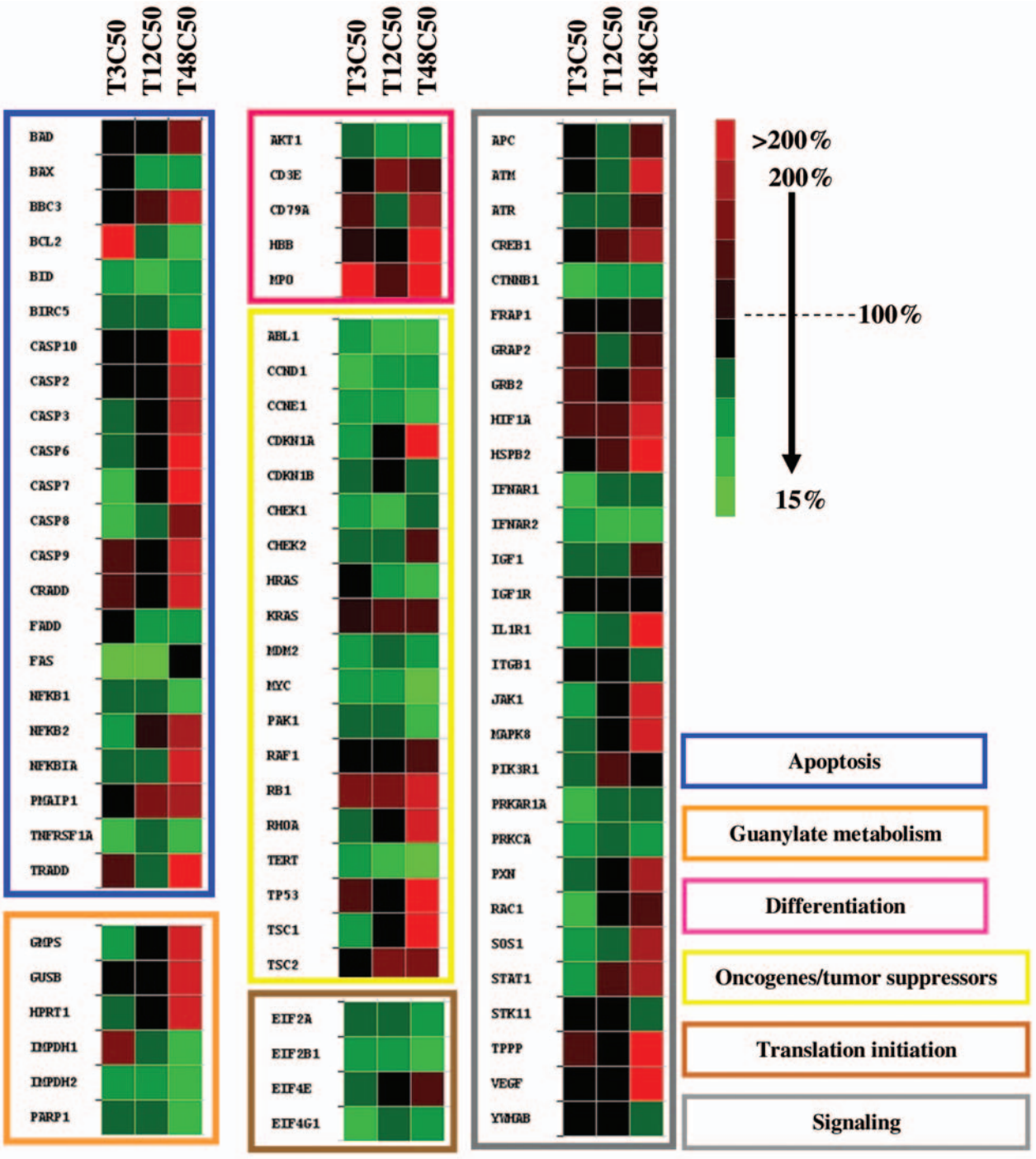

Figure 4. *Expression profiles of the tested genes grouped according to biological role. The expression of 85 genes were determined in K562 cells treated with 50 μM Ribavirin. Each colour patch in the resulting visual map represents the gene expression level with a continuum from dark green (lowest) to bright red (highest) as indicated. T3, T12 and T48 stand for treatment timepoints in hours. C50 means 50 μM drug concentration. SE values were <10% .*

There are previous reports on the different behaviour of normal and cancer cells upon exposure to Ribavirin that may underlie the agent's anticancer effects (9, 14, 34). The gene expression pattern of normal blood cells was not influenced by Ribavirin exposure (19). It is concluded that, apart from its well-established role as an antiviral agent and an IMPDH inhibitor, Ribavirin by itself can fundamentally influence the global gene expression pattern of K562 leukemia cells,

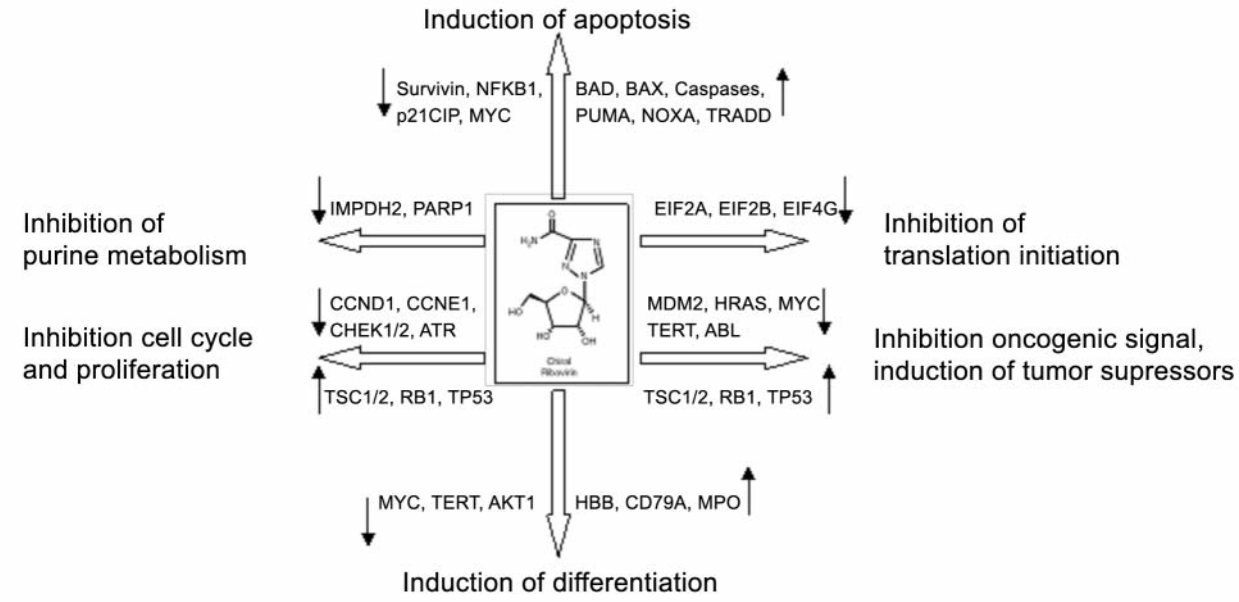

Figure 5. *Schematic representation of anticancer effects of Ribavirin. The main effects of the drug involve induction of both apoptosis and differentiation. Ribavirin can act against leukemia cells through inhibition of the following processes: cell proliferation, oncogenic signaling, expression of genes involved in purine biosynthesis and translation initiation. In addition it up-regulates several tumor suppressors. The direction of the expression changes that are supposed to be responsible for the effects of Ribavirin is indicated by arrows (down-regulation or up-regulation).*

affecting not only translation initiation but also inducing the apoptotic and differentiation programs as well as modifying several other cancer-related processes. Taken together, these results are well in line with the rationale of the initiation of the Ribavirin treatment trial which started recently on AML patients (www.ribatrial.com).

Further studies are needed to identify the key changes at the protein level that are responsible for the drug's action and to determine whether these findings can be generalized to other cell types.

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