women undergoing ART. In contrast, other authors propose that thrombophilia screening in IVF in the general population is not cost-effective (Fàbregues *et al*, 2004).

It is important to recognize that thrombosis, as a complication of ovarian stimulation or OHSS, is an increasing problem. As the use of ART increases, the occurrence of related thromboembolic complications must also be investigated to determine their causes. Clearly, further research is needed to identify women at risk and to develop adequate therapeutic and prophylactic measures.

Acknowledgements

We are indebted to Prof. W. H. Stone for his help in the revision and discussion of the manuscript. This work was supported in part by Red Cardiovascular RECAVA (RD 06/0014/0016) from Instituto de Salud Carlos III (Ministerio de Sanidad y Consumo) and by Departament d'Innovació, Universitats i Empresa from Generalitat de Catalunya (2005 SGR 0168).

E. Martí[1]
A. Santamaría[1]
J. Mateo[1]
A. Tolosa[1]
L. Querol[2]
P. Viscasillas[3]
J. Fontcuberta[1]

[1]*Haemostasis and Thrombosis Unit, Departments of Haematology,* [2]*Neurology, and* [3]*Obstetrics and Gynaecology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.*
E-mail: emartis@santpau.es

References

Aboulghar, M.A., Mansour, R.T., Serour, G.I. & Amin, Y.M. (1998) Moderate ovarian hyperstimulation syndrome complicated by deep cerebrovascular thrombosis. *Human Reproduction*, **13,** 2088–2091.

Aune, B., Hoie, K.E., Oian, P., Holst, N. & Osterud, B. (1991) Does ovarian stimulation for in-vitro fertilization induce a hypercoagulable state? *Human Reproduction*, **6,** 925–927.

Di Micco, P., D'Uva, M., Romano, M., Di Micco, B. & Niglio, A. (2003) Stroke due to left carotid thrombosis in moderate ovarian hyperstimulation syndrome. *Thrombosis and Haemostasis*, **90,** 957–960.

Dulitzky, M., Cohen, S., Inbal, A., Seidman, D.S., Soriano, D., Lidor, A., Masiach, S. & Ravinovici, J. (2002) Increased prevalence of thrombophilia among women with severe ovarian hyperstimulation syndrome. *Fertility and Sterility*, **77,** 463–467.

Fàbregues, F., Tàssies, D., Reverter, J.C., Carmona, F., Ordinas, A. & Balasch, J. (2004) Prevalence of thrombophilia in women with severe ovarian hyperstimulation syndrome an cost-effectiveness of screening. *Fertility and Sterility*, **81,** 989–995.

Grandone, E., Colaizzo, D., Vergura, P., Cappucci, F., Vecchione, G., Lo Bue, A., Cittadini, E. & Margaglione, M. (2004) Age and homocysteine levels are risk factors for thrombotic complications after ovarian stimulation. *Human Reproduction*, **19,** 1796–1799.

Vlahos, N.F. & Gregoriou, O. (2006) Prevention and management of ovarian hyperstimulation syndrome. *Annals of the New York Academy of Sciences*, **1092,** 247–264.

Keywords: thrombosis, thrombophilia, *in vitro* fertilization, ovarian hyperstimulation syndrome.

First published online 19 March 2008
doi:10.1111/j.1365-2141.2008.07120.x

IPO-trimethylation of histone H3-lysine^{9} associated with P210 BCR-ABL tyrosine kinase of chronic myeloid leukaemia

BCR-ABL is the molecular hallmark and the causative event of chronic myeloid leukaemia (CML). Deregulated proliferation and extended survival of leukaemic progenitors are mostly driven by multiple interactions of its p210 kDa protein tyrosine kinase (TK) with downstream signalling pathways (Melo & Deininger, 2004). However, the regulatory mechanisms of BCR-ABL expression remain elusive. Gene transcription is governed by covalent post-translational modifications of core histone N-terminal residues (including acetylation, methylation and phosphorylation) that provide binding platforms for specific regulatory proteins of DNA-mediated events. Accordingly, enzymes that catalyse those modifications, including histone acetyl-transferases (HDACs) and histone methyl-transferases (HMTs), have a key role in transcriptional repression or activation. We recently demonstrated that BCR-ABL expression was regulated by global lysine (K) hyper-acetylation and K^{20} ipo-trimethylation associated with p210 TK (Brusa *et al*, 2006). Previous studies showed that H4-K^{20} trimethylation was preceded by H3-K^{9} trimethylation induced by a specific HMT, SUV39H1, contributing with HDAC1, 2 and 3 to heterochromatin protein 1 (CBX5, HP1) recruitment to chromatin and transcriptional repression (Schotta *et al*, 2004; Stewart *et al*, 2005). The present study aimed to elucidate the impact of p210 BCR-ABL TK activity on H3-K^{9} methylation status. To this purpose, we used a 32D cell clone (8B) expressing a temperature sensitive (ts) BCR-ABL construct (whose proteins shows TK activity at the optimum temperature of 33°C, but not at the non-optimum temperature of 39°C) and

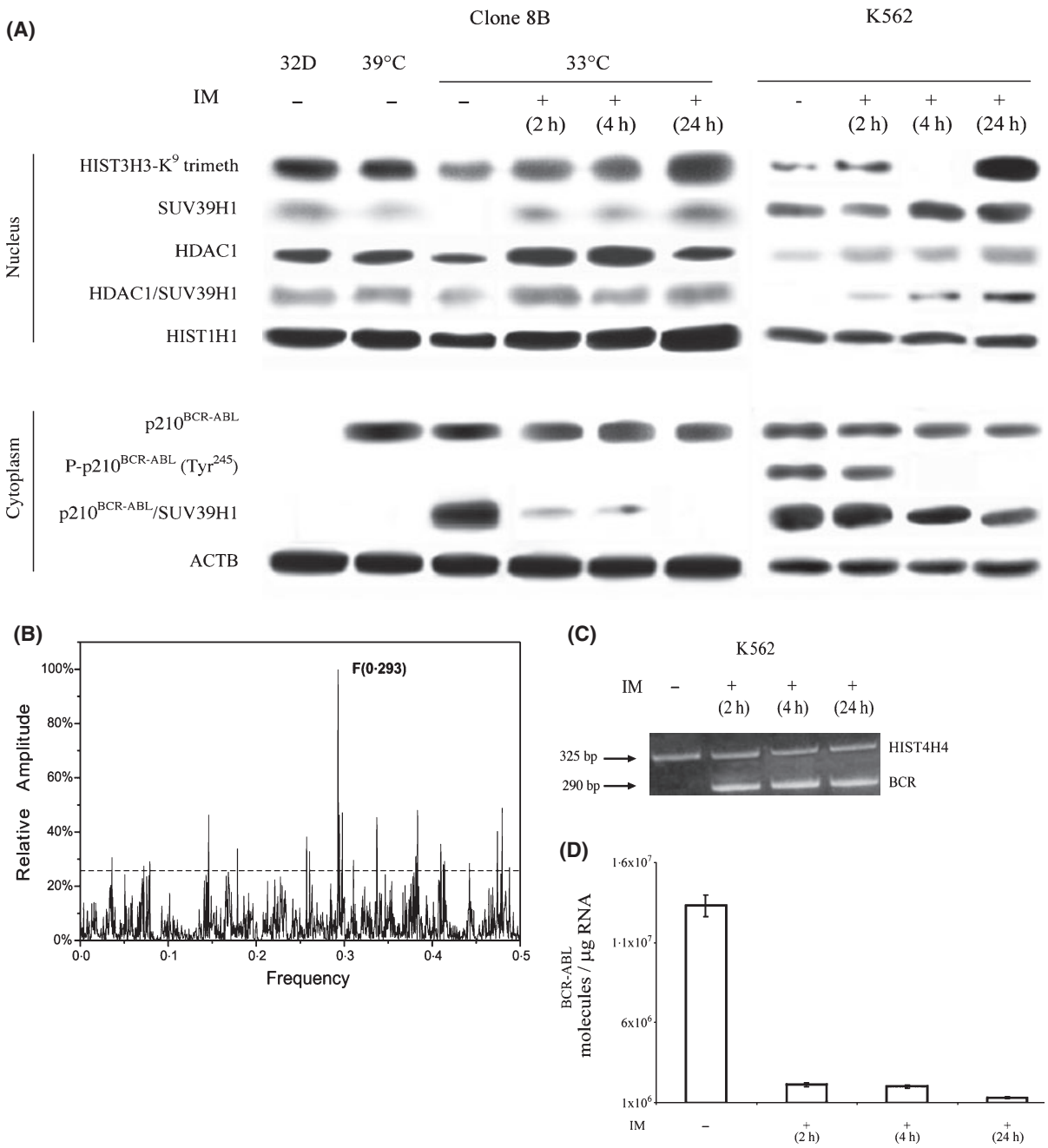

Fig 1. H3-K^9 ipo-trimethylation associated with p210 BCR-ABL TK arises from the cytoplasmatic compartmentalization of SUV39H1 bound to the fusion protein. (A) The impact of p210 BCR-ABL TK on H3-K^9 methylation status and SUV39H1 subcellular location was investigated in 32D cell clone (8B) stably transduced with a ts BCR-ABL mutant construct kept at the optimum (33°C) temperature for fusion protein TK activity and in K562 cell line. Parental 32D cell line and clone 8B kept at the non permissive temperature (39°C) served as controls for lacking BCR-ABL expression and p210 TK activity, respectively. (B) Cross spectrum (CS) between p210 BCR-ABL and SUV39H1. The abscissa axis indicates the frequencies from the Fourier transform of the sequence of electron-ion interaction potential corresponding to the amino-acid sequence of the protein. The ordinate axis indicates the amplitudes (in arbitrary units) corresponding to each frequency component in the spectrum. The dashed line represents positive control value. (C) PCR amplification of a BCR promoter region critical for BCR-ABL transcription performed on DNA from anti-SUV39H1 ChIP products of K562 cell line provides evidence for SUV39H1 recruitment at BCR promoter between 2 and 24 h of exposure to 1 µmol/l imatinib mesylate (IM). The constitutively acetylated promoter of human histone H4a (region −40 to +285) was used as internal control for the amplification reaction. D-BCR-ABL expression in K562 cell line was measured by competitive PCR. The ordinate axis indicates the number of BCR-ABL transcript molecules/microg total RNA. The housekeeping gene (*GAPDH*) did not exhibit any difference relative to p210 BCR-ABL TK activity (data not shown).

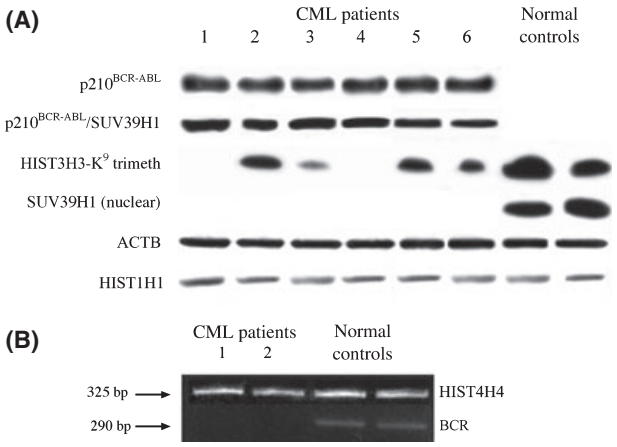

Fig 2. H3-K^9 trimethylation status and SUV39H1 subcellular location in early CD34$^+$ haematopoietic progenitors. (A) CD34$^+$ cells were purified from bone marrow samples of six CML patients at diagnosis and peripheral blood aphaeresis of two haematologically normal persons (intended for bone marrow transplantation) after informed consent. Eighty per cent or more of CD34$^+$ cells from CML patients exhibited BCR-ABL based on FISH analysis (data not shown). (B) PCR amplification of a BCR promoter region critical for BCR-ABL transcription performed on DNA from anti-SUV39H1 ChIP products of CD34$^+$ from CML patients no. 1 and 2 and normal controls. See legend to Fig 1 for details.

K562 cell line (Campanini *et al*, 2001). Using a highly specific antibody (Abcam, Cambridge, UK) H3-K^9 trimethylation in clone 8B at 33°C was shown to be significantly lower compared to the 32D parental cell line (not expressing *BCR-ABL1*) and clone 8B at 39°C (lacking p210 TK activity) (Fig 1A). Exposure of clone 8B at 33°C and K562 to the TK inhibitor Imatinib mesylate (IM at 1 μmol/l concentration) induced a significant and persistent increase of H3-K^9 trimethylation, confirming H3-K^9 ipo-trimethylation association with p210 BCR-ABL TK activity (Fig 1A).

H3-K^9 ipo-trimethylation results from SUV39H1 'loss of function' due to its improper subcellular location. Co-immunoprecipitation/immunoblotting experiments using anti-ABL SH2 and anti-SUV39H1 antibodies [from Upstate Biotechnology (Lake Placid, NY, USA) and Santa Cruz Biotech (Santa Cruz, CA, USA) respectively] showed that SUV39H1 was confined to the cytoplasm bound to p210 BCR-ABL TK (Fig 1A). Its nuclear importation both in clone 8B at 33°C and K562 followed p210 BCR-ABL de-phosphorylation in response to IM exposure (Fig 1A). Within the nucleus SUV39H1 was coupled to HDAC1, confirming the two enzyme physical interaction in a so-called 'histone deacetylase complex' (Fig 1A) (Vaute *et al*, 2002).

The informational spectrum method (ISM) is a virtual spectroscopy method for protein structural and functional analyses (see *Supplementary material* for details), which identified one dominant peak in the cross-spectrum (CS) of p210 BCR-ABL and SUV39H1 at 0·293 Fourier frequency, confirming the two protein mutual interaction (Fig 1B) (Veljkovic *et al*, 2007). *In silico* peptide mapping provided evidence for the involvement of SUV39H1 SET domain in p210 BCR-ABL interaction. Notably, the SUV39H1 N-terminus domain required for the enzyme interaction with HDAC1 does not participate in its ligand binding to p210 BCR-ABL TK (see Fig S1 and S2) (Vaute *et al*, 2002).

The putative role of SUV39H1 recruitment to chromatin in BCR-ABL transcription was investigated in K562 cells, where BCR-ABL expression is under the control of BCR promoter (ts BCR-ABL expressed through transfection in clone 8B is under the control of an heterologous promoter: The myeloproliferative sarcoma virus LTR promoter). Polymerase chain reaction (PCR) amplification of DNA from chromatin immuno-precipitation products (ChIP) with the anti-SUV39H1-ChIP grade antibody (Upstate Biotechnology) demonstrated that SUV39H1 was recruited at a BCR promoter critical for BCR-ABL transcription after p210 BCR-ABL TK inhibition by IM (Fig 1C). SUV39H1 tethering to chromatin is expected to repress transcription through mechanisms involving CBX5 (HP1) stable recruitment to trimethylated H3-K^9 and histone deacetylation (Stewart *et al*, 2005). Accordingly, the number of BCR-ABL transcript molecules (quantified by mean of a previously published competitive PCR strategy) underwent a significant reduction between 2 and 24 h of IM exposure (Fig 1D) (Brusa *et al*, 2006).

H3-K^9 methylation status and SUV39H1 subcellular location relative to BCR-ABL expression were further investigated in early myeloid progenitors (CD34$^+$) obtained from bone marrow samples of six CML patients at diagnosis and peripheral blood apheresis of two haematologically normal persons intended for autologous transplantation after informed consent. Preliminary experiments (not shown) did not determine differences in histone acetylation and methylation patterns and BCR-ABL expression relative to CD34$^+$ source in CML patients. CD34$^+$ cell concentration following immuno-magnetic sorting was 95% or greater and more than 80% of CD34$^+$ cells from CML patients harboured *BCR-ABL1*, based on fluorescence *in situ* hybridization analysis, and expressed the p210 fusion protein (data not shown and Fig 2A). H3-K^9 trimethylation was significantly lower in CD34$^+$ cells from four CML patients compared to CD34$^+$ normal subjects and completely absent in the other two (Fig 2A). Moreover, SUV39H1 was undetectable in the nuclear compartment of CD34$^+$ cells from all CML patients: It was located in the cytoplasm bound to p210 BCR-ABL (Fig 2A). Further investigation is required to elucidate whether H3-K^9 tri-methylation, still apparent in CD34$^+$ cells of CML patients 2, 3, 5 and 6 in spite of SUV39H1 absence in the nuclear compartment, arises from enzymatic activities of other HMTs involved in H3-K^9 trimethylation, including SUV39H2 and ESET/SETDB1 (Kouzarides, 2007). PCR amplification performed on DNA from anti-SUV39H1 ChIP products of two CML patients (patients 1 and 2) and normal persons confirmed that, in BCR-ABL-expressing CD34$^+$ cells, SUV39H1 is not recruited to the BCR promoter (Fig 2B).

In conclusion, our work confirms an epigenetic control of BCR-ABL expression involving H3-K^9 ipo-trimethylation as a

result of SUV39H1 cytoplasmatic compartmentalization by p210 BCR-ABL TK.

SUV39H1 association with HDAC1, following its release from the cytoplasmatic ligand to p210 BCR-ABL fusion protein TK and nuclear import in response to IM, may reinforce the repressive chromatin state and influence BCR-ABL expression.

Manuela Mancini[1]
Elisa Zuffa[1]
Nevena Veljkovic[2]
Gianluca Brusa[1]
Patrizia Corrado[1]
Valentina Corradi[3]
Giovanni Martinelli[1]
Enza Barbieri[4]
Maria Alessandra Santucci[1]

[1]*Istituto di Ematologia e Oncologia Medica "Lorenzo e Ariosto Seràgnoli", University of Bologna-Medical School, Bologna, Italy,* [2]*Centre for Multidisciplinary Research, Institute of Nuclear Sciences VINCA, Belgrade, Serbia,* [3]*Dipartimento Farmaco Clinico Tecnologico, University of Siena, Siena, and* [4]*Istituto di Radioterapia "Luigi Galvani", University of Bologna-Medical School, Bologna, Italy.*
E-mail: mancini_manu@yahoo.com

Acknowledgements

The study was supported by University of Bologna (ex 60% funds), Bologna*AIL* and Carisbo Foundation.

References

Brusa, G., Zuffa, E., Mancini, M., Benvenuti, M., Calonghi, N., Barbieri, E. & Santucci, M.A. (2006) P210 Bcr-abl tyrosine kinase interaction with histone deacetylase 1 modifies histone H4 acetylation and chromatin structure of chronic myeloid leukemia haematopoietic progenitors. *British Journal of Haematology*, **132,** 359–369.

Campanini, F., Santucci, M.A., Pattacini, L., Brusa, G., Piccioli, M., Barbieri, E., Babini, L. & Tura, S. (2001) Competitive polymerase chain reaction to detect the amplification of bcr-abl gene of chronic myeloid leukemia. *Haematologica*, **86,** 167–173.

Kouzarides, T. (2007) Chromatin modifications and their function. *Cell*, **128,** 693–705.

Melo, J.V. & Deininger, M.W (2004) Biology of chronic myelogenous leukemia signaling pathways of initiation and transformation. *Hematology/Oncology Clinics of North America*, **18,** 545–568.

Schotta, G., Lachner, M., Sarma, K., Ebert, A., Sengupta, R., Reuter, G., Reinberg, D. & Jenuwein, T. (2004) A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes & Development*, **18,** 1251–1262.

Stewart, M.D., Li, J. & Wong, J. (2005) Relationship between histone H3 lysine 9 methylation, transcription repression, and heterochromatin protein 1 recruitment. *Molecular and Cellular Biology*, **25,** 2525–2538.

Vaute, O., Nicolas, E., Vandel, L. & Trouche, D. (2002) Functional and physical interaction between the histone methyltransferase Suv39H1 and histone deacetylases. *Nucleic Acids Research*, **30,** 475–481.

Veljkovic, V., Veljkovic, N., Esté, J.A., Huther, A. & Dietrich, U. (2007) Application of the EIIP/ISM bioinformatics concept in development of new drugs. *Current Medicinal Chemistry*, **14,** 441–453.

Supplementary material

The following supplementary material is available for this article online:

Fig S1. CS between p210 BCR-ABL TK and SUV39H1-△SET; the abscissa axis indicates the frequencies from the Fourier transform of the sequence of electron–ion interaction potential corresponding to the amino-acid sequence of the protein.

Fig S2. CS between p210 BCR-ABL TK and SUV39H1-△N180. See legend to Fig S1 for details.

The material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2141.2008.07134 (This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Keywords: chronic myeloid leukaemia, p210 BCR-ABL tyrosine kinase, histone covalent modifications, SUV39H1, gene transcription.

First published online 28 March 2008
doi:10.1111/j.1365-2141.2008.07134.x

JAK2 V617F mutation is associated with increased risk of thrombosis in Chinese patients with essential thrombocythaemia

Essential thrombocythaemia (ET) is a Philadelphia-chromosome–negative myeloproliferative disorder (MPD) characterized by persistent thrombocytosis, excessive proliferation of megakaryocytes in the bone marrow, normal erythrocytic mass, and the absence of prominent bone marrow fibrosis. The *JAK2* V617F mutation, caused by a G>T transversion in exon