CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Archivio istituzionale della ricerca - Università di Palermo

In Vitro Cell.Dev.Biol.—Animal (2014) 50:48–55
DOI 10.1007/s11626-013-9676-3

Cytogenetic characterization of HB2 epithelial cells from the human breast

Fabio Caradonna · Claudio Luparello

Received: 2 July 2013 / Accepted: 25 July 2013 / Published online: 27 August 2013 / Editor: T. Okamoto
© The Society for In Vitro Biology 2013

Abstract HB2 is a cell line originated by subcloning of MTSV1-7 mammary luminal epithelial cells isolated from human milk and immortalization *via* introduction of the gene encoding *simian virus 40* (*SV40*) *large T antigen*. Despite its wide utilization as non-neoplastic counterpart in assays aimed to elucidating various biochemical and genetical aspects of normal and tumoral breast cells, to our knowledge no literature data have so far appeared concerning the chromosomal characterization of the HB2 cells. Here, we report the cytogenetic characterization of the karyotype of HB2 cells, which puts in evidence the occurrence of changes in chromosomal number and structure and the presence of unidentified chromosomal markers in variable amount. Our results do not detract from the utility of HB2 cells in illustrating fundamental aspects of breast cell biology, but rather interject a note of caution into generalizing results obtained with this cell line to other non-immortalized epithelial cell populations from the human breast. Therefore, this work represents a useful resource for all who want to perform appropriate and focused future studies on this cell line and proposes precise indications for a knowledgeable use of HB2 cells.

Keywords Human breast · HB2 cells · G-banded karyotype · Jumping translocation

Introduction

HB2 is a cell line originated by subcloning of MTSV1-7 mammary luminal epithelial cells isolated by Bartek *et al.* (1991) from human milk and immortalized by introducing the gene encoding *simian virus 40* (*SV40*) *large T antigen* (Berdichevsky et al. 1994). HB2 cells were selected for their ability to undergo branching morphogenesis from spherical structures when cultured in collagen gels and stimulated by hepatocyte growth factor and used as a cell model system for the study of the involvement of fibroblast-derived growth factor, Wnt, and integrins in mammary tissue development (Huguet et al. 1995; Alford et al. 1998). Subsequently, HB2 cells have been used as non-neoplastic counterpart for elucidating various biochemical and genetical aspects of normal and tumoral breast cell biology, such as Wnt5a- and heregulin-stimulated signal transduction pathways (*e.g.*, Baeckström et al. 2000a; Jönsson and Andersson 2001; Dejmek et al. 2003), c-ERBb2 promotion of apoptosis and effect on *MUC1* expression (*e.g.*, Baeckström et al. 2000b; Scibetta et al. 2001), ethanol promotion of cell invasion (*e.g.*, Aye et al. 2004), cadmium-mediated changes in gene expression (Sirchia et al. 2008; Sirchia and Luparello 2009), *TP53* transcriptional regulation (Goes et al. 2011), and epigenetic modulation of oncosuppressor genes (Rubinek et al. 2012).

It is widely acknowledged that the process of transformation and immortalization of cell lines *via* SV40 T antigen may cause genetic hypervariability and genomic instability with induction of chromosomal aberration and aneuploidy, probably due to the binding of the viral oncoprotein to components of the mitotic spindle checkpoint, such as Bub1, and consequent genomic destabilization (Ray et al. 1990; Cotsiki et al. 2004). Despite their ever-growing utilization as "normal" control in parallel with breast cancer cell lines in assays aimed to evaluate several different features of neoplastic cell behavior to our knowledge, no literature data have so far appeared concerning the chromosomal characterization of the HB2 cells in order to gain information on possible anomalies concerning the number and structure of the chromosomes. In the present paper, we report the cytogenetic characterization of the karyotype of HB2 cells, which puts in evidence the occurrence of changes in chromosomal number and structure and the

F. Caradonna (✉) · C. Luparello
Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), University of Palermo, Edificio 16, Viale delle Scienze, 90128 Palermo, Italy
e-mail: fabio.caradonna@unipa.it

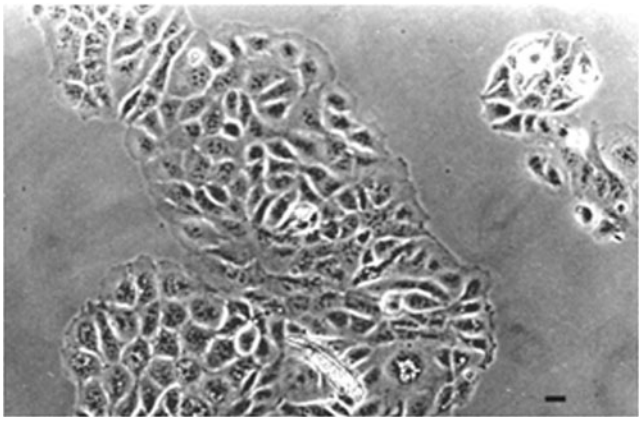

Figure 1. Phase contrast micrograph showing the morphological appearance of HB2 cells in flask. The cultures show an epithelial phenotype with polygonal cells growing strictly connected in scattered islands. *Bar* 10 μM.

presence of unidentified chromosomal markers in variable amount.

Materials and Methods

Cell culture. The HB2 breast epithelial cell line (courtesy of Cancer Research, UK) was obtained in 2007. Cells were cultured in high glucose–DMEM medium plus 10% fetal calf serum (Life Technologies, Carlsbad, CA), 5 μg hydrocortisone/ml (Sigma, St. Louis, MO), 10 μg bovine insulin (Sigma), and antibiotic/antimycotic mixture (100 U/ml penicillin, 100 μg/ml streptomycin, and 2.5 mg/l amphotericin B; Life Technologies) at 37°C in a 5% CO_2 atmosphere. No test for the presence of mycoplasma contamination was performed during the assays. Cell morphology was checked under the phase contrast microscope (Fig. 1). For subsequent experiments, exponentially growing cells were exposed to 0.1 μg colcemid (Sigma)/ml for 2 h and harvested by trypsinization.

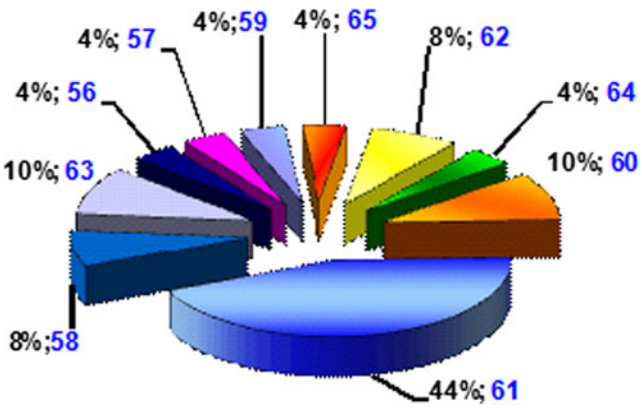

Figure 2. Graph showing the karyotypic chromosome number distribution (in percent) in HB2 cells. The modal karyotype has 61 chromosomes.

Metaphase chromosomal spread preparation and cytogenetic analysis. Cytogenetic analyses were performed according to Sciandrello *et al.* (2002). Trypsinized HB2 cells were collected by centrifugation at 800 rpm for 8 min submitted to hypotonic treatment with 75 mM KCl for 20 min at 37°C, and subsequently fixed with two changes of Carnoy's solution

Table 1. Cytogenetic analysis of 25 selected GTG-banded HB2 metaphases

N	Karyotype[a]	*UMAR*(*s*)[b]
1	56XX, +der(3), +3p-, +4, +5, der(6), +7, +9, +12, +13, +16, +20, +20	0
1	57XX, +der(1), der(3), +3q-, +5, +7, +9, +12, +13, +16, +20, +20	+2
1	58XX, +der(1), +der(3), +3q-, +4, +5, +der(7), +12, +13, +15	+3
1	58XX, +der(1), +der(3), +3q-, der(6), +12, +13, +15, +16	+5
1	59XX, +der(1), +der(3), +3q-, der(6), +der(7), +9, +12, +13, +16	+5
1	60XX, +der(1), der(3), +3q-, +5, +7, +der(7), +12, +15, +20, +22	+5
1	60XX, +der(1), der(3), +3p-, +3q-,+5, +7, +der(7), +13, +15, +20	+5
1	61XX, +der(1), +der(3), +4, der(6), +7, +7, +9, +12, +13, +16, +20	+5
1	61XX, +der(1), +3p-, +3q-,+4, +5, +6, +7, +der(7), +11, +14, +20, +22	+3
1	61XX, +der(1), +der(3), +5, der(6), +7, +7, +9, +12, +13, +16, +20	+5
1	61XX, +der(1), +der(3), +3p-, +5, +6, +7, +der(7), +11, +12, +14, +20, +22	+3
1	61XX, +der(3), +3p-, +4 +5, +6, +7, +der(7), +11, +12, +14, +20, +22	+3
1	61XX, +der(1), +der(3), +3q-, +5, +6, +7, +der(7), +12, +14, +15, +20, +22	+3
1	61XX, +der(1), +der(3), +3q-, +5, der(6), +7, +9, +12, +13, +16, +20	+6
1	61XX, +der(1), der(3), +3q-, +5, +7, +der(7), +9, +12, +13, +16, +20	+6
1	61XX, +der(1), der(3), +3p-, +3q-, +5, +7, +der(7), +12, +13, +15, +20, +22	+4
2	61XX, +der(1), der(3), +3q-, der(6), +7, +der(7), +9, +12, +13, +16, +20	+6
1	62XX, +der(1), der(3), +3p-, +4, +5, der(6), +7, +9, +12, +16, +20, +20	+6
1	62XX, +der(1), der(3), +3p-, +3q-, +5, +7, +der(7), +9, +12, +13, +16, +20	+6
1	63XX, +der(3), +3p-, +5, +6, +7, +der(7), +11, +12, +14, +15, +20, +22	+5
1	63XX, +der(1), der(3), +3q-, +4, +5, der(6), +7, +9, +12, +13, +16, +20, +20	+6
1	63XX, +der(1), +der(3), +5, +6, +7, +der(7), +14, +15, +20, +22	+7
1	64XX, +der(1), der(3), +3q-, +4, +5, +6, +7, +der(7), +11, +12, +13, +14, +15, +20, +22	+4
1	65XX, +der(1), +der(3), +3p-, +3q-,+5, +7, +der(7), +12, +13, +15, +20, +22	+7

[a] According to ISCN (2013)

[b] Unidentified marker(s)

(3:1 mixture of methanol/acetic acid) at room temperature, dropped onto chilled slides, air dried and stained with 2.5% Giemsa. A hundred metaphases were scored and three independent experiments were performed to determine the frequency of cells with different numbers of chromosomes. GTG banding was performed by incubating the glass slides in a 0.04% trypsin solution (Life Technologies) for 7 s, followed by rinsing the slides in phosphate-buffered saline buffer and staining in a 2% Giemsa solution in Sorensen's phosphate buffer, pH 6.8, for 8 min. The slides were rinsed with water and air dried. Twenty-five GTG-banded metaphases were examined to determine the karyotype.

Image acquisition. Metaphases were visualized under a Microphot microscope equipped with a charge-coupled device camera and Genikon image analysis software (Nikon Instruments, Sesto Fiorentino, Italy).

Results

In a first set of assays, the modal chromosome number was determined by analysis of 100 Giemsa-stained metaphases. As shown in the graph in Fig. 2, most metaphase spread preparations were approximately less than triploid with a predominant fraction, accounting for 44% of samples, endowed with 61 chromosomes. Then, HB2 cell karyotype was studied by analysis of 25 GTG-banded metaphase spreads, selected among those showing a number of chromosomes close or equal to the modal value (range, 56–65). The cytogenetic analysis (Table 1) puts in evidence the presence of identifiable numerical and structural chromosomal changes, as well as unidentified chromosomal markers in variable amount (Figs. 3, 4). In particular, the trisomy of chromosomes 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, 16, 20, and 22 and the tetrasomy of chromosomes 7 and 20 were observed with a frequency comprised between 20% and 86.7%, and 6.7% and 13.3%, respectively (Fig. 5). Structural alterations were observed in chromosomes 1, 3, 6, and 7 with a frequency comprised between 40% and 93.3% (Fig. 6). In particular, in the 86.7% of the analyzed metaphase spread preparations chromosome 1 appeared involved in a rearrangement leading to the appearance of a longer chromosomal marker, der(1), due to supernumerary bands in the short arm. Such marker was always found as an additional structure, thereby generating chromosome 1 trisomy (Fig. 7*A*). GTG band analysis allowed to identify the complex rearrangement leading to the appearance of the der(1) marker consisting in two inversions and one duplication event of the short arm. The identification of the rearrangement-related break points allowed to describe this chromosomal marker (Fig. 7*B*) as inv(1)(p22p33), dup(1)(p33→p36), inv(1)(p33p36).

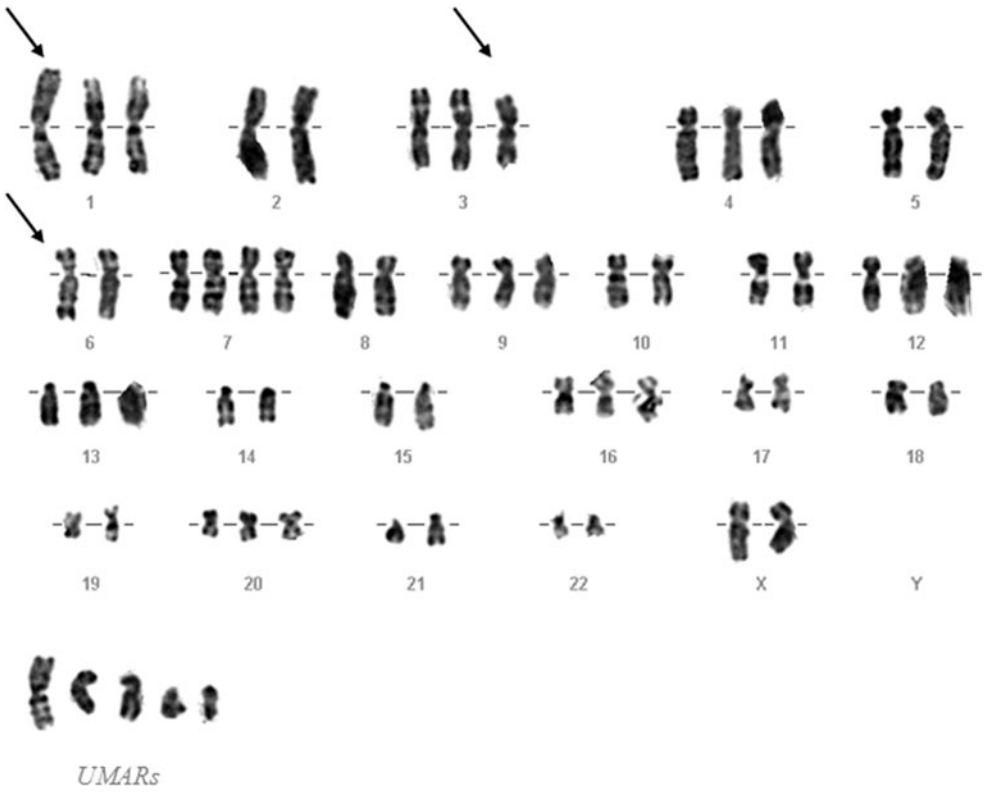

Figure 3. GTG-banded karyotype of an HB2 cell: 61XX, +der(1), +der(3), +4, der(6), +7, +7, +9, +12, +13, +16, +20, +5 unidentified markers (*UMARs*). The *arrows* indicate the abnormal chromosomes.

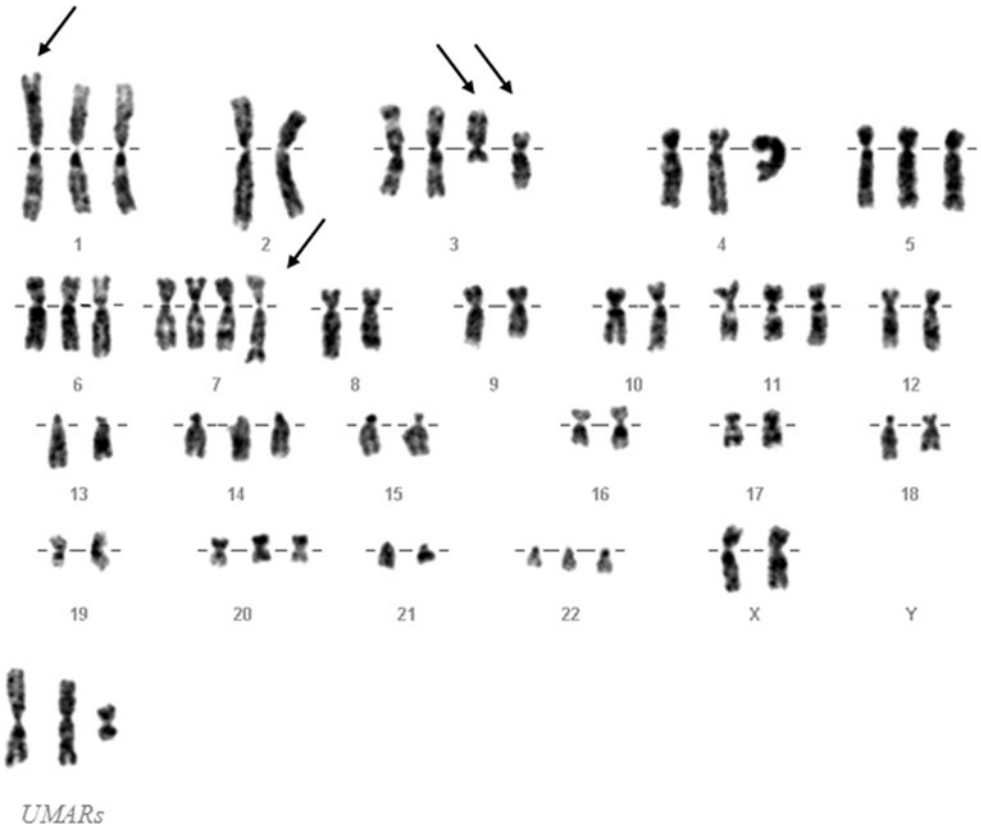

Figure 4. GTG-banded karyotype of an HB2 cell: 61XX, +der(1), +3p-, +3q-,+4, +5, +6, +7, +der(7), +11, +14, +20, +22, +3 unidentified markers (*UMARs*). The *arrows* indicate the abnormal chromosomes.

Interestingly, chromosome 3 underwent to two different types of rearrangement each one generating a distinct chromosomal marker. In fact, in 93.3% of analyzed metaphase spread preparations, this chromosome was involved in a "jumping translocation" with either chromosomes 6 or 7 leading to the appearance of der(3), der(6), and der(7) markers (Fig. 8). The identified break points for this translocation were located in the short arm of chromosome 3 (in the region 3p24-p23) and in the long arm of chromosome 6 (in 6q25 band) and chromosome 7 (in the region 7q31-q36). The der(3) marker was found to be an additional structure to chromosomes 3 in 40% of samples, thereby generating a partial chromosome 3 trisomy. The second type of rearrangement was a t(3;20) generating the chromosomal markers 3p- and 3q- (Fig. 9). The identified break points for this translocation were located in the centromeric regions of the two involved chromosomes. In addition, most of the metaphases showing this translocation were

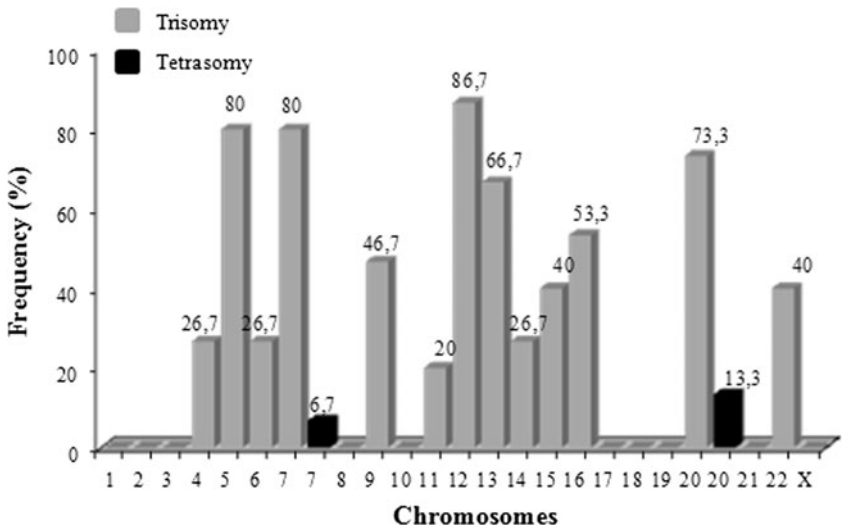

Figure 5. Histograms showing the distribution of aneuploidy in HB2 cells.

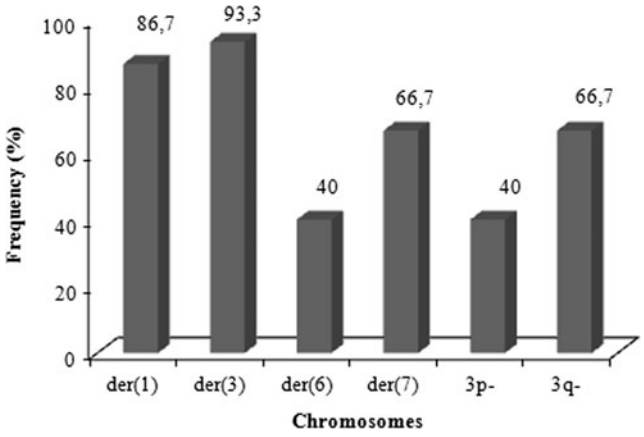

Figure 6. Histograms showing the distribution of structural abnormalities in HB2 cells.

characterized by partial chromosome 3 trisomy and also chromosome 20 tetrasomy.

Discussion

The development of immortalized cell lines is a useful approach to identify the genetic and biological events associated with proliferation, differentiation, and de-differentiation in human tissues. In fact, immortalized cells resemble their primary cell counterparts but have the advantage of being carried through long-term culture as well as being cost-effective and derived from the same donor. On the other hand, it is widely acknowledged that expression of viral oncogenes in cells results in a number of genotypic and phenotypic changes (*e.g.*, Dimri et al. 2005). In the present paper, we show that karyotypic analysis of HB2 cells reveals frequent aneuploidy and chromosomal abnormalities, as expected for SV40-transformed breast epithelial cells (Toouli et al. 2002). In particular, the occurrence of chromosomal rearrangements leading to the generation of der(1), der(3), der(6), and der(7) was reported. Concerning der(1), translocations events in 1p22 have been described as the most usually involved in the onset of human cancer (Sandberg 1980; Elco et al. 2010). The 1p22 band contains a fragile site which was shown to be inducible by aphidicolin in cultured human lymphocytes (Tedeschi et al. 1992) and this break point was commonly found in the karyotype of liver cancer cells (Wong et al. 2000). Interestingly, 1p22 band contains the *dihydropyrimidine dehydrogenase* gene, which encodes an initial and rate-limiting enzyme acting in the uracil and thymine catabolic pathways and being responsible for degradation of 5-fluorouracil, which is also considered as a potential prognostic marker for breast cancers (Li et al. 2004). Moreover, 1p33 band contains the *cyclin-dependent kinase inhibitor 2C* gene encoding p18 of the INK4 family which functions as a cell cycle regulator that controls G_1 progression *via* inhibition of CDK4 (Guan et al. 1994), whereas the *cell division cycle 2-like 2* (*PITSLRE protein*) *variant 3* gene, encoding a member of the subfamily of $p34^{cdc2}$-related protein kinases which intervene in the regulation of apoptosis, and at least other three potential tumor suppressor genes (*i.e.*, *Heir-1*, *TNFR*, and *DAN*) are located in band 1p36 which contains another aphidicolin-inducible

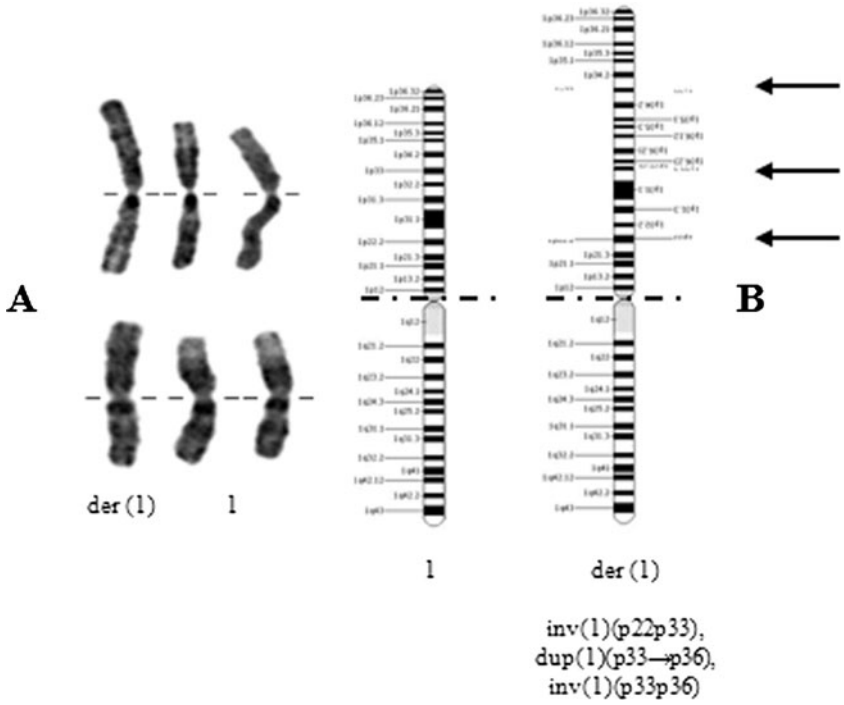

Figure 7. *A*, Partial GTG-banded karyotype of two HB2 cells showing a rearranged chromosome 1 compared to the normal ones. *B*, Ideogram of der(1) compared to the normal chromosome 1; the arrows indicate the breakpoints.

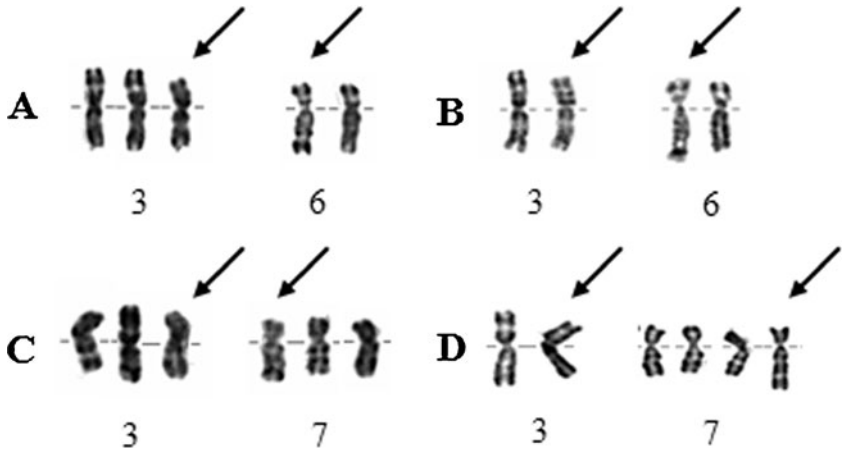

Figure 8. Partial GTG-banded lkaryotype of four HB2 cells showing jumping translocation between chromosomes 3 and 6 (*A–B* breakpoints in 3p24-p23 region and in 6q25 band) and between chromosomes 3 and 7 (*C–D* breakpoints in 3p24-p23 and in 7q31-q36 regions). The *arrows* indicate the abnormal chromosomes.

chromosomal fragile site (Lahti et al. 1995; Thompson et al. 1997; Tunca et al. 2000).

"Jumping translocations" are rare events described for the first time by Lejeune *et al.* (1979) characterized by the presence of a common chromosomal segment, originated by a donor chromosome on two or more different acceptor chromosomes. These translocations have been observed in SV40-immortalized cell lines and in patients with constitutive or acquired chromosomal alterations (Jackson-Cook et al. 2003; Berger and Bernard 2007). The breakpoints of the jumping translocation identified in HB2 cells are located in 3p24-p23 and 7q31-q36 regions and in 6q25 band. This could lead to a deregulation of *RNA binding motif*, *single-stranded interacting protein*, and *transcript variant 3* (RBMS3) gene, present in 3p24 band, whose product is an RNA-binding protein that belongs to the *c-myc* gene single-strand binding protein family and is involved in the regulation of cell cycle and apoptosis (Chen et al. 2012). On the other hand, 6q25 band contains the *F-box protein 5* gene that encodes an homologue of a *X. laevis* protein, named EMI1, acting as a mitotic regulator *via* interaction with Cdc20 and inhibition of the anaphase-promoting complex (Reimann et al. 2001), as well as other cell division regulatory genes such as *gravin* (AKAP12) and *AT-rich interactive domain 1B variant 1* (Nagl et al. 2005; Canton et al. 2012). Several proliferation-related genes are also located in 7q31-q36 region, including *CDC26*, *inhibitor of growth family*, *member 3*, and the proto-oncogene *MET* (see Hembase, http://fmp-8.cit.nih.gov/hembase/chr.php?chr=7, accessed 28 May 2013).

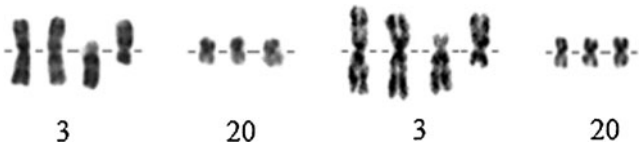

Figure 9. Partial GTG-banded karyotype of two HB2 cells showing the chromosomal markers 3p- and 3q- originated by t(3; 20)

The translocation t(3;20) originates from breakages occurring in the centromeric regions of the two involved chromosomes. It is known that centromere-related sequences are commonly heterochromatic; nonetheless, some genes controlling cell life/death are located closely to the break points and could conceivably be deregulated after the translocation event. As an example, molecular alterations of *ROBO1* and *-2* genes, mapping into 3p12 band, are known to be linked to carcinogenesis (Ghosh et al. 2009), whereas *zinc finger protein 133* and *protein tyrosine phosphatase*, *receptor type*, *T* genes, located in 20p11.23 and 20q12-q13 regions, respectively, are recognized regulators of cell growth, differentiation, mitotic cycle, and oncogenic transformation (Lee et al. 2007; Scott and Wang 2011).

Conclusions

In conclusion, the data here reported indicate that although HB2 cells show a relatively unaltered epithelial phenotype with polygonal cells growing strictly connected in scattered islands when cultured in flask (see Fig. 1), their immortalization process produced a number of genomic alterations, here described for the first time, which must be taken into account when designing experiments and choosing the cell model systems. In addition, this cell line shows a relative morphologic and genomic stability as demonstrated by serial cytogenetic analyses (data not shown) performed up to 10 months during its expanded growth in our laboratory in which we found no significant deviations of the numerical/structural chromosome changes.

Our present results do not detract from the utility of HB2 cells that can illustrate many fundamental aspects of breast cell biology and are useful in their own right, but rather interject a note of caution into extrapolating results obtained with this cell line used as the "normal" counterpart of neoplastic breast cells when comparing patterns of gene

expression, or cellular behavior, since their unique properties might not be generalized. Therefore, this work represents a useful resource for all who want to perform appropriate and focused future studies on this cell line by either molecular genetic or molecular cytogenetic techniques. The conventional cytogenetic findings here reported and the hypotheses of gene involvement here proposed give precise indications for a knowledgeable use of HB2 cells.

Acknowledgments HB2 cells are courtesy of Cancer Research (UK). This work was supported by grants from the University of Palermo (ex60% and FFR).

The authors wish to dedicate this work to the cytogeneticist Paolo Carbone, who has recently celebrated his twentieth death anniversary. Being right, he always was an advocate of the big potential of conventional cytogenetics as an indispensable basic analysis to knowingly perform every further molecular study.

References

Alford D.; Baeckström D.; Geyp M.; Pitha P.; Taylor-Papadimitriou J. Integrin-matrix interactions affect the form of the structures developing from human mammary epithelial cells in collagen or fibrin gels. *J Cell Sci* 111: 521–532; 1998.

Aye M. M.; Ma C.; Lin H.; Bower K. A.; Wiggins R. C.; Luo J. Ethanol-induced *in vitro* invasion of breast cancer cells: the contribution of MMP-2 by fibroblasts. *Int J Cancer* 112: 738–746; 2004.

Baeckström D.; Alford D.; Taylor-Papadimitriou J. Morphogenetic and proliferative responses to heregulin of mammary epithelial cells *in vitro* are dependent on HER2 and HER3 and differ from the responses to HER2 homodimerisation or hepatocyte growth factor. *Int J Oncol* 16: 1081–1090; 2000a.

Baeckström D.; Lu P. J.; Taylor-Papadimitriou J. Activation of the alpha2beta1 integrin prevents c-erbB2-induced scattering and apoptosis of human mammary epithelial cells in collagen. *Oncogene* 19: 4592–45603; 2000b.

Bartek J.; Bartkova J.; Kyprianou N.; Lalani E. N.; Staskova Z.; Shearer M.; Chang S.; Taylor-Papadimitriou J. Efficient immortalization of luminal epithelial cells from human mammary gland by introduction of simian virus 40 large tumor antigen with a recombinant retrovirus. *Proc Natl Acad Sci U S A* 88: 3520–3524; 1991.

Berdichevsky F.; Alford D.; D'Souza B.; Taylor-Papadimitriou J. Branching morphogenesis of human mammary epithelial cells in collagen gels. *J Cell Sci* 107: 3557–3568; 1994.

Berger R.; Bernard O. A. Jumping translocations. *Genes Chromosome Cancer* 46: 717–723; 2007.

Canton D. A.; Keene C. D.; Swinney K.; Langeberg L. K.; Nguyen V.; Pelletier L.; Pawson T.; Wordeman L.; Stella N.; Scott J. D. Gravin is a transitory effector of polo-like kinase 1 during cell division. *Mol Cell* 48: 547–559; 2012.

Chen J.; Kwong D. L.; Zhu C. L.; Chen L. L.; Dong S. S.; Zhang L. Y.; Tian J.; Qi C. B.; Cao T. T.; Wong A. M.; Kong K. L.; Li Y.; Liu M.; Fu L.; Guan X. Y. RBMS3 at 3p24 inhibits nasopharyngeal carcinoma development *via* inhibiting cell proliferation, angiogenesis, and inducing apoptosis. *PLoS One* 7: e44636; 2012.

Cotsiki M.; Lock R. L.; Cheng Y.; Williams G. L.; Zhao J.; Perera D.; Freire R.; Entwistle A.; Golemis E. A.; Roberts T. M.; Jat P. S.; Gjoerup O. V. Simian virus 40 large T antigen targets the spindle assembly checkpoint protein Bub1. *Proc Natl Acad Sci U S A* 101: 947–952; 2004.

Dejmek J.; Dib K.; Jönsson M.; Andersson T. Wnt-5a and G-protein signaling are required for collagen-induced DDR1 receptor activation and normal mammary cell adhesion. *Int J Cancer* 103: 344–351; 2003.

Dimri G.; Band H.; Band V. Mammary epithelial cell transformation: insights from cell culture and mouse models. *Breast Cancer Res* 7: 171–179; 2005.

Elco C. P.; Marino-Enriquez A.; Abraham J. A.; Dal Cin P.; Hornick J. L. Hybrid myxoinflammatory fibroblastic sarcoma/hemosiderotic fibrolipomatous tumor: report of a case providing further evidence for a pathogenetic link. *Am J Surg Pathol* 34: 1723–1727; 2010.

Ghosh S.; Ghosh A.; Maiti G. P.; Alam N.; Roy A.; Roychoudhury S.; Panda C. K. Alterations of ROBO1/DUTT1 and ROBO2 loci in early dysplastic lesions of head and neck: clinical and prognostic implications. *Hum Genet* 125: 189–198; 2009.

Goes A. C.; Cappellen D.; Santos Jr. G. C.; Pirozhkova I.; Lipinski M.; Vassetzky Y.; de Moura-Gallo C. V. Loop domain organization of the p53 locus in normal and breast cancer cells correlates with the transcriptional status of the TP53 and the neighboring genes. *J Cell Biochem* 112: 2072–2081; 2011.

Guan K. L.; Jenkins C. W.; Li Y.; Nichols M. A.; Wu X.; O'Keefe C. L.; Matera A. G.; Xiong Y. Growth suppression by p18, a p16INK4/MTS1- and p14INK4B/MTS2-related CDK6 inhibitor, correlates with wild-type pRb function. *Genes Dev* 8: 2939–2952; 1994.

Huguet E. L.; Smith K.; Bicknell R.; Harris A. L. Regulation of Wnt5a mRNA expression in human mammary epithelial cells by cell shape, confluence, and hepatocyte growth factor. *J Biol Chem* 270: 12851–12856; 1995.

ISCN 2013: An International System for Human Cytogenetic Nomenclature. Recommendations of the International Standing Committee on Human Cytogenetic Nomenclature. Published in collaboration with 'Cytogenetic and Genome Research' Plus fold-out: 'The Normal Human Karyotype G- and R-bands'. Editor(s): Shaffer L.G., McGowan-Jordan J., Schmid M.

Jackson-Cook C.; Zou Y.; Turner K.; Astbury C.; Ware J. A novel tumorigenic human prostate epithelial cell line (M2205): molecular cytogenetic characterization demonstrates C-MYC amplification and jumping translocations. *Cancer Genet Cytogenet* 141: 56–64; 2003.

Jönsson M.; Andersson T. Repression of Wnt-5a impairs DDR1 phosphorylation and modifies adhesion and migration of mammary cells. *J Cell Sci* 114: 2043–2053; 2001.

Lahti J. M.; Xiang J.; Heath L. S.; Campana D.; Kidd V. J. PITSLRE protein kinase activity is associated with apoptosis. *Mol Cell Biol* 15: 1–11; 1995.

Lee S. J.; Lee J. R.; Hahn H. S.; Kim Y. H.; Ahn J. H.; Bae C. D.; Yang J. M.; Hahn M. J. PIAS1 interacts with the KRAB zinc finger protein, ZNF133, *via* zinc finger motifs and regulates its transcriptional activity. *Exp Mol Med* 39: 450–457; 2007.

Lejeune J.; Maunoury C.; Prieur M.; Van den Akker J. A jumping translocation (5p;15q), (8q;15q), and (12q;15q). *Ann Genet* 22: 210–213; 1979.

Li H.; Suo Z.; Zhang Y.; Risberg B.; Karlsson M. G.; Villman K.; Nesland J. M. The prognostic significance of thymidine phosphorylase, thymidylate synthase and dihydropyrimidine dehydrogenase mRNA expressions in breast carcinomas. *Histol Histopathol* 19: 129–136; 2004.

Nagl Jr. N. G.; Patsialou A.; Haines D. S.; Dallas P. B.; Beck Jr. G. R.; Moran E. The p270 (ARID1A/SMARCF1) subunit of mammalian SWI/SNF-related complexes is essential for normal cell cycle arrest. *Cancer Res* 65: 9236–9244; 2005.

Ray F. A.; Peabody D. S.; Cooper J. L.; Cram L. S.; Kraemer P. M. SV40 T antigen alone drives karyotype instability that precedes neoplastic transformation of human diploid fibroblasts. *J Cell Biochem* 42: 13–31; 1990.

Reimann J. D.; Freed E.; Hsu J. Y.; Kramer E. R.; Peters J. M.; Jackson P. K. Emi1 is a mitotic regulator that interacts with Cdc20 and inhibits the anaphase promoting complex. *Cell* 105: 645–655; 2001.

Rubinek T.; Shulman M.; Israeli S.; Bose S.; Avraham A.; Zundelevich A.; Evron E.; Gal-Yam E. N.; Kaufman B.; Wolf I. Epigenetic silencing of the tumor suppressor klotho in human breast cancer. *Breast Cancer Res Treat* 133: 649–657; 2012.

Sandberg A. A. The chromosomes in human cancer and leukemia. Elsevier, New York, pp 567–596; 1980.

Sciandrello G.; Barbaro R.; Caradonna F.; Barbata G. Early induction of genetic instability and apoptosis by arsenic in cultured Chinese hamster cells. *Mutagenesis* 17: 99–103; 2002.

Scibetta A. G.; Albanese I.; Morris J.; Cooper L.; Downward J.; Rowe P. P.; Taylor-Papadimitriou J. Regulation of MUC1 expression in human mammary cell lines by the c-ErbB2 and ras signaling pathways. *DNA Cell Biol* 20: 265–274; 2001.

Scott A.; Wang Z. Tumour suppressor function of protein tyrosine phosphatase receptor-T. *Biosci Rep* 31: 303–307; 2011.

Sirchia R.; Longo A.; Luparello C. Cadmium regulation of apoptotic and stress response genes in tumoral and immortalized epithelial cells of the human breast. *Biochimie* 90: 1578–1590; 2008.

Sirchia R.; Luparello C. Short-term exposure to cadmium affects the expression of stress response and apoptosis-related genes in immortalized epithelial cells from the human breast. *Toxicol In Vitro* 23: 943–949; 2009.

Tedeschi B.; Vernole P.; Sanna M. L.; Nicoletti B. Population cytogenetics of aphidicolin-induced fragile sites. *Hum Genet* 89: 543–547; 1992.

Thompson F. H.; Taetle R.; Trent J. M.; Liu Y.; Massey-Brown K.; Scott K. M.; Weinstein R. S.; Emerson J. C. Band 1p36 abnormalities and t(1;17) in ovarian carcinoma. *Cancer Genet Cytogenet* 96: 106–110; 1997.

Toouli C. D.; Huschtscha L. I.; Neumann A. A.; Noble J. R.; Colgin L. M.; Hukku B.; Reddel R. R. Comparison of human mammary epithelial cells immortalized by simian virus 40 T-antigen or by the telomerase catalytic subunit. *Oncogene* 21: 128–139; 2002.

Tunca B.; Egeli U.; Zorluoğlu A.; Yilmazlar T.; Yerci O.; Kizil A. The expression of fragile sites in lymphocytes of patients with rectum cancer and their first-degree relatives. *Cancer Lett* 152: 201–209; 2000.

Wong N.; Lai P.; Pang E.; Wai-Tong Leung T.; Wan-Yee Lau J.; Johnson P. J. A comprehensive karyotypic study on human hepatocellular carcinoma by spectral karyotyping. *Hepatology* 32: 1060–1068; 2000.