Dyskerin expression influences the level of ribosomal RNA pseudo-uridylation and telomerase RNA component in human breast cancer

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Abstract
Dyskerin is a nucleolar protein, altered in dyskeratosis congenita, which carries out two separate functions, both fundamental for proliferating cells. One function is the pseudo-uridylation of ribosomal RNA (rRNA) molecules, necessary for their processing, and the other is the stabilization of the telomerase RNA component, necessary for telomerase activity. A significant feature of dyskeratosis congenita is an increased susceptibility to cancer; so far, however, no data have been reported on dyskerin changes in human tumours. Therefore, in this study, the distribution of dyskerin in a large series of human tumours from the lung, breast, and colon, as well as from B-cell lymphomas, was analysed by immunohistochemistry. Dyskerin proved never to be lost or delocalized outside the nucleolus. A quantitative analysis of dyskerin mRNA expression was then performed in 70 breast carcinomas together with the evaluation of telomerase RNA component levels and rRNA pseudo-uridylation. Dyskerin mRNA levels were highly variable and directly associated with both telomerase RNA component levels and rRNA pseudo-uridylation. Dyskerin gene silencing in the MCF-7 human breast carcinoma cell line reduced telomerase activity and rRNA pseudo-uridylation. Significantly, patients with low dyskerin expression were characterized by a better clinical outcome than those with a high dyskerin level. These data indicate that dyskerin is not lost in human cancers and that the levels of its expression and function are associated with tumour progression.

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Introduction
In humans, point mutations in the DKC1 gene cause the rare skin, mucosal, and bone marrow failure syndrome termed X-linked dyskeratosis congenita (DC) [1]. The DKC1 product, dyskerin, is a nucleolar protein which has at least two different functions. First, dyskerin is a component of small nucleolar ribonucleoprotein particles (RNPs) crucial for rRNA processing. In particular, dyskerin is involved in the conversion of specific uridine residues to pseudo-uridine and is thus considered a putative pseudo-uridylase of H/ACA RNPs [1,2]. The second function is to stabilize the telomerase enzymatic complex through the binding of a specific sequence in the telomerase RNA component (hTR) [3]. The alterations of dyskerin result, on the one hand, in both the reduction of rRNA pseudo-uridylation and the slowing down of ribosome rRNA processing rate [4,5] and, on the other hand, in the degradation of the telomerase RNA component and in the impairment of the enzymatic activity of the telomerase complex [3,5]. The alterations in both ribosome biogenesis and telomerase function can explain the reduced proliferative capacity observed in DC patients. However, one of the salient features of X-linked DC is an increased susceptibility to cancer. Before the age of 30, approximately 8–10% of DC patients develop tumours, mostly originating from the skin, the gastrointestinal mucosa, and the bone marrow [6].

It is not clear what specific function of dyskerin must be impaired in order to contribute to tumorigenesis. The absence of telomerase function may lead to an increased susceptibility to cancer, as is observed in mice lacking telomerase activity due to the deletion of the telomerase RNA component [7]. In this model, telomere attrition — which is functionally evident only after crossing the hTR knockout mice for five to six generations — leads to a moderate increase in the...
incidence of spontaneous tumours in late-generation mice, probably due to chromosomal instability [8]. On the other hand, an increased incidence of tumours has been observed in a hypomorphic mouse in which dyskerin is targeted and its expression is reduced to approximately 30% of normal levels [4] (the DKC1 knockout is embryonic lethal [8]). In this mouse, the reduced expression of dyskerin results in a reduction of both telomerase activity and rRNA pseudo-uridylation. Interestingly, in the DKC1 hypomorphic mice, the increase in tumour incidence — mainly lung adenocarcinomas, B-cell lymphomas, and breast cancers — is also observed in the early generations, when telomeres are still very long. Therefore, it has been concluded that defective pseudo-uridylation of rRNA could play a role in promoting tumourigenesis [4].

Altogether, data obtained in DC patients and in DKC1m hypomorphic mice indicate that dyskerin may behave as a tumour suppressor, thus promoting cancer when not functioning properly. A crucial question that remains unanswered is whether a reduction in dyskerin expression or a change in its function may promote cancer not only in the rare X-linked DC and in experimental models, but also in human cancer. In this scenario, a fraction of cases from various kinds of human tumours should be characterized by either the loss or the functional impairment of dyskerin. Therefore, in a first study, we evaluated by immunohistochemistry whether dyskerin was lost or delocalised outside the nucleus in a series of human primary tumours of various histological origins. In all the tumours analysed, we detected the presence of dyskerin protein localized in the nucleolus. In a second study, we investigated dyskerin expression and function quantitatively in a series of human breast carcinomas, as this tumour type is frequent in mice characterized by reduced dyskerin expression and function [4]. Dyskerin mRNA levels were highly variable and positively related with both hTR and rRNA pseudo-uridylation levels, thus suggesting that the levels of dyskerin expression might influence both telomerase activity and rRNA pseudo-uridylation. We therefore investigated whether the reduction of dyskerin expression can actually impair hTR stabilization, telomerase activity, and proper ribosome processing in breast cancer cells. For this purpose, we selectively reduced DKC1 mRNA levels using RNA interference in the MCF-7 human breast cancer cell line. Lastly, data on dyskerin expression and function obtained on breast carcinomas were compared with the available clinico-pathological parameters.

Materials and methods

Immunohistochemical analysis of dyskerin expression in human tumours of different origins

We generated a hamster anti-dyskerin polyclonal serum by repeated administration of affinity-purified recombinant dyskerin protein. Dyskerin staining was performed with the obtained serum diluted 1:100 by utilizing an automated staining machine (Discovery XT Staining Module, Ventana Medical Systems Inc, Tucson, AZ, USA). Anti-nucleolin (Stressgene, San Diego, CA, USA) and anti-nucleophosmin (Zymed Inc, San Francisco, CA, USA) primary monoclonal antibodies were also used.

The study cohort comprised 115 non-small cell lung carcinomas, 90 breast carcinomas, 102 colon carcinomas, and 78 B-cell lymphomas diagnosed at the Memorial Sloan-Kettering Cancer Center from 1984 through 2000. Patient anonymity was ensured and the study received a waiver by the Institutional Review Board (WA-0323-04). Biopsies were classified histologically in accordance with the World Health Organization classification. Tissue micro-arrays (TMAs) were constructed in the Pathology and Molecular Cytology Core Facilities as previously described [9].

Evaluation of mRNA expression and functional activity of dyskerin in human breast cancer

Patients

A total of 70 carcinomas of the breast were studied. Cases were selected from a series of 211 consecutive patients who underwent surgical resection for primary infiltrating carcinomas of the breast at the Surgical Department of the University of Bologna between 1994 and 1995, only on the basis of frozen tissue availability. The study was approved by the Senior Staff Committee, a board regulating non-interventional studies at the time of patients’ enrolment comparable to an Institutional Review Board. Tumours were histologically classified according to the World Health Organisation criteria. Histological grading was performed according to Elston and Ellis [10]. Tumour size was evaluated in freshly obtained tissue, before formalin fixation, and coded according to the UICC pT recommendations. Axillary node status was assessed by pathological staging after axillary node dissection. Due to patient age, axillary dissection was not performed in two cases.

Immunohistochemical analysis and nucleolar silver-staining

Immunohistochemical evaluation was performed on routinely processed tissue samples using a streptavidin–biotin–peroxidase complex protocol. The following monoclonal antibodies were used: anti-RB protein (clone G3-245, which specifically recognizes the phosphorylated form of RB protein), anti-Ki67 (clone MIB-1), anti-oestrogen receptor (anti-ER; clone 1D5), anti-progesterone receptor (anti-PGR; clone 1A6), anti-p53 (clone BP53-12.1), and anti-p27 (clone DCS72), all from BioGenex Laboratories (San Ramon, CA, USA). Nuclear immunostaining was assessed using the Cytometria program (C&V, Bologna, Italy), as previously detailed [11], and expressed as the percentage of labelled nuclear area over the total neoplastic nuclear area in the section (labelling index area: LIa). For each case, at least 2000 cells were evaluated.
Nucleolar silver-staining was performed as previously reported [12]. For each case, at least 200 cells were evaluated.

Real-time RT-PCR
Total RNA was extracted from frozen samples using Trizol reagent (Invitrogen, Darmstadt, Germany). For each sample, 10 µg of total RNA was reverse-transcribed using a High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA), following the manufacturer’s instructions. The cDNA was subjected to real-time PCR analysis in a Gene Amp 7000 Sequence Detection System (Applied Biosystems) using the TaqMan approach. Cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles at 95°C for 15 s, and 60°C for 1 min. For each sample, three replicates were analysed. Sets of primers and fluorogenic probes specific for dyskerin and TERT mRNA were purchased from Applied Biosystems (Assay on Demand), while hTR-specific primer and probe were synthesized as previously described [13]. The relative amounts of the studied target genes were calculated using the expression of human glyceraldehyde-3-phosphate dehydrogenase or β-glucuronidase as an endogenous control (Applied Biosystems). The final results were determined as follows: N target = 2^(-ΔΔCt sample−ΔΔCt calibrator), where the ΔΔCt values of the sample and calibrator were determined by subtracting the Ct value of the endogenous control gene from the Ct value of each target gene. As calibrator, the value obtained using a cDNA preparation from the SW48 cell line was used.

rRNA pseudo-uridine evaluation
Forty micrograms of total RNA was electrophoresed on a 1.2% agarose–2.2 M formaldehde gel. Bands corresponding to 28S and 18S rRNA were cut from the gel and RNA was purified using DNA ultra-free spin columns (Millipore Corporation, Billerica, MA, USA), isopropanol-precipitated, and resuspended in sterile water. The 18S and 28S rRNAs obtained were separately treated with 20 U of RNAse T2 (Sigma–Aldrich, St Louis, MO, USA) for 1 h at 37°C after the addition of 0.4 vol of 50 mM Tris-base and 0.1 vol of 10 mM MgCl2. The final volume of this reaction was 100 µl. The resulting nucleotides were then dephosphorylated by incubating with 1 Unit of alkaline phosphatase (Fluka, Sigma-Aldrich, St Louis, MO, USA) for 1 h at 37°C after the addition of 0.4 vol of 50 mM Tris-base and 0.1 vol of 10 mM MgCl2. The final volume of this reaction was 100 µl. The resulting nucleosides were then subjected to high-performance liquid chromatographic separation in a Beckman System Gold Programmable Solvent Module 126 equipped with a detector Module 166 set at 254 nm (Beckman-Coulter, Fullerton, CA, USA). The column (0.39 x 30 cm) was a reversed-phase µBondapak C18 (particle size 10 µm) purchased from Waters Associates (Milford, MA, USA). As previously described [14], mobile phase conditions were 0.1 M phosphate buffer (pH 6)/methanol, 99 : 1 (v/v) for 12 min, 96 : 4 (v/v) for 13 min, and 85 : 15 (v/v) for 25 min. Pseudo-uridine and major nucleosides used as standards were purchased from Berry and Associates, Inc (Dexter, MI, USA).

DKC1 mRNA knockdown using siRNAs
Asynchronously growing MCF-7 cells were subjected to RNA interference (RNAi) for DKC1 mRNA. Cells were grown in standard conditions and transfected with three different DKC1 siRNA sequences or with scrambled sequences matched for GC content as a negative control (Stealth RNAi, Invitrogen, Carlsbad, CA, USA). Transfections were performed utilizing Lipofectamin 2000 by following procedures recommended by the manufacturer (Invitrogen).

Evaluation of telomerase activity
Telomerase activity in cell lines or tissue samples was measured by using the Telomerase PCR ELISA kit (Roche Applied Sciences, Milano, Italy) according to the manufacturer’s protocol. For each condition, 0.5 and 1 µg of cell or tissue extract was analysed. After the initial incubation step, the elongation products were amplified by PCR for 29 cycles.

Statistical analysis
Correlations between continuous variables were computed by means of the Spearman rank-correlation coefficient. Differences among groups were evaluated using the Mann–Whitney U- or Kruskal-Wallis test, as appropriate. Univariate analysis for disease-free survival (DFS) was performed using the Kaplan and Meier approach [15], and the differences between curves were tested using the log-rank test. Multivariate analysis was conducted according to the Cox proportional hazards regression model [16].

Results
Immunohistochemical evaluation of dyskerin in human cancer tissue micro-arrays
The use of anti-dyskerin polyclonal serum for standard western blot analysis resulted in a single specific band at 57 kD, corresponding to the expected molecular weight for dyskerin [1], when tested on lysates of human origin (Figure 1a). Immunohistochemical staining resulted in specific nucleolar labelling, showing the same localization and distribution pattern as other proteins that are known to localize to the nucleolus (ie nucleophosmin, nucleolin) (Figure 1a). Immunohistochemical analysis was then performed on TMAs generated from human tumours of different histological origins. TMAs from lung (115 cases), breast (90 cases), and colon (102 cases) cancers and from
Dyskerin in breast cancer

Figure 1. Immunohistochemical analysis of dyskerin in human tumours. (a) The use of the anti-dyskerin serum for standard western blot analysis on PAGE separated lysates derived from the human H1299 non-small-cell lung carcinoma cell line resulted in a single specific band at 57 kD, corresponding to the expected molecular weight of dyskerin. Immunohistochemical detection with anti-dyskerin serum resulted in the same morphological pattern obtained with nucleolar markers such as nucleophosmin (NPM) and nucleolin. (b–d) Representative staining with anti-dyskerin antibody on lung (b), breast (c), and colon (d) carcinomas. Each panel shows two representative tumours of the same histological type characterized by different dyskerin expression (low or high). Although the intensity of the staining was variable, dyskerin was always detectable in the samples tested. Original magnifications: ×600

B-cell lymphomas (78 cases) were tested with anti-dyskerin serum. In all cases, dyskerin was present and localized in the nucleoli of cancer cells, in general; however, variable intensity of the staining was consistent with highly variable dyskerin expression among samples (Figures 1b–1d).

Evaluation of dyskerin mRNA expression and dyskerin function in breast cancer

Immunohistochemistry demonstrated that dyskerin is not lost or completely delocalized outside the nucleolus in human tumours, a finding consistent with previous observations indicating that dyskerin is necessary for the survival of proliferating cells [8]. Moreover, immunohistochemistry showed that dyskerin expression is extremely variable among samples. For this reason, since the quantitative variations of dyskerin levels cannot be precisely evaluated by means of immunohistochemistry, dyskerin mRNA was measured by real-time PCR on a series of infiltrating breast carcinomas. The mean relative value of the 70 samples tested was 1.61 (range 0.04–4.54; SD 0.88; median value 1.45).

To understand whether major dyskerin functions were affected by different dyskerin mRNA levels, the
Our results suggest that impairment of hTR stabilization and of proper ribosome processing occurs in tumours expressing low amounts of dyskerin. In order to study the effect of the reduction of dyskerin expression in breast cancer cells under controlled conditions, we selectively knocked down dyskerin mRNA levels by RNA interference in the MCF-7 breast cancer cell line. The mean value of the two evaluations is reported. Forty-eight hours after transfection of DKC1-specific siRNAs, the levels of dyskerin mRNA were significantly reduced compared with MCF-7 cells transfected with control scrambled sequences. This effect was maintained for as long as 96 h after transfection (see Figure 4a). Reduction of the levels of dyskerin mRNA generates a concomitant steep reduction of hTR levels as well as of telomerase activity (see Figures 4a and 4b). Moreover, HPLC analysis of rRNA demonstrated that dyskerin mRNA knockdown reduces pseudo-uridylation. Indeed, the rRNA ψ/C ratio of cells transfected with DKC1-specific siRNAs is reduced for 18S RNA and, to a lesser extent, for 28S RNA (see Figures 4a and 4c) compared with controls.

Association between dyskerin mRNA expression and other clinicopathological parameters in breast cancers

Our results on tumour specimens indicate that in the samples studied, dyskerin mRNA levels are variable and significantly associated with hTR levels and rRNA pseudo-uridylation. Moreover, our RNAi experiments show that in breast cancer cells, the reduction of dyskerin levels impairs telomerase activity and alters rRNA pseudo-uridylation. These two events are known to be related to the clinical behaviour of tumours. In order to investigate the significance of reduced dyskerin expression and function on tumour progression, we compared dyskerin mRNA expression data with the clinical and bio-pathological parameters commonly used to assess the clinical outcome of breast cancer patients. These included age, pT and N status, histological grade, ER and PGR status, Ki67 LIa, p53 LLa, p27 LLa, and RB LLa. Nucleolar size was also evaluated as a marker of the rapidity of cell proliferation [19,20]. No significant association was found among dyskerin expression and pT and N status and histological and nuclear grade, considered as categorical variables. Linear regression analysis showed that dyskerin expression was not significantly related to age, ER and PGR status, Ki67 LLa, p53 LLa, p27 LLa, and RB LLa. Nucleolar size, considered as a continuous variable.

Follow-up data were available for 62 patients (mean follow-up = 81 months, range 4–115 months). On the basis of dyskerin mRNA level, we arbitrarily divided the cases into three groups: namely, group 1, with low dyskerin values ($n = 25$, dyskerin relative values $\leq 1.32$); group 2, with intermediate dyskerin values ($n = 22$, dyskerin values between 1.32 and 2.18); and group 3, with high dyskerin values ($n = 15$, dyskerin relative values $\geq 2.18$), choosing the cut-offs which allowed us to obtain the highest predictive

Figure 2. hTR levels and dyskerin mRNA expression in breast carcinomas. Box plot of hTR relative expression obtained in samples with low and high dyskerin mRNA expression. hTR levels appear to be decreased in tumours with low dyskerin mRNA expression ($p = 0.001$). The cut-offs used to distinguish low and high dyskerin mRNA expression are the same as those used between groups 1 and 2 in the survival curves (see text and Figure 5).
Figure 3. HPLC analysis of pseudo-uridylation in rRNA derived from breast carcinomas. This analysis allowed us to distinguish the four major nucleosides and $\psi$ from tumour-derived 18S and 28S rRNAs. (a) Standard chromatogram with $\psi$ (1 pmol) and U (15 pmol) nucleosides. (b) Complete chromatogram from digested and dephosphorylated 18S rRNA (8 $\mu$g). From the injection time five major peaks are detected, corresponding to $\psi$, C, U, G, and A nucleosides, respectively. The retention times observed are in line with studies already described (see ref 13). (c, d) Significant $\psi$ and C peaks obtained with 18S and 28S rRNAs in a tumour with low dyskerin mRNA expression (c, sample relative expression of dyskerin mRNA = 0.61) and a tumour with high dyskerin expression (d, sample relative expression of dyskerin mRNA = 2.09). (e) Box plots of the $\psi$/C peak area ratio of 28S and 18S rRNA obtained in samples with low and high dyskerin mRNA expression. Pseudo-uridylation is reduced in tumours with low dyskerin expression ($p = 0.008$) and 28S ($p = 0.01$) rRNA The cut-off used to distinguish low and high dyskerin mRNA expression is the same as that used between groups 1 and 2 in the survival curves (see text and Figure 5).

We also evaluated the predictive value of hTR levels in our series. No association was found between DFS and hTR levels using different cut-offs. However, we noticed that within the 25 samples of the group characterized by low dyskerin values, the hTR levels were significantly related to DFS. DFS analysis showed that the sub-group of eight patients with the lowest hTR values was characterized by an extremely unfavourable prognosis (DFS rate = 37.5%), while in the remaining 17 cases, no recurrence or death was recorded (DFS rate = 100%; $\chi^2 = 15.02, p = 0.0001$). Interestingly, no difference in value. Disease-free survival (DFS) analysis performed using the log-rank test showed that group 1 tumours were characterized by a higher (80%) DFS rate than tumours of group 2 (68.18%), which, in turn, had a higher DFS rate than group 3 (40%; $\chi^2 = 7.98, p = 0.0185$; Figure 5). Therefore, low dyskerin levels appear to be associated with a more favourable outcome. Moreover, dyskerin expression proved to be the only independent factor ($p = 0.0091$) when tested by multivariate analysis with those parameters showing a significant association with DFS in our series, namely nucleolar size and Ki67 LIa.
Figure 4. Effect of dyskerin reduction in the MCF-7 breast carcinoma cell line. (a) The RNAi approach allowed us to decrease dyskerin mRNA levels in the MCF-7 human breast carcinoma cell line to 13% and 12% of control cells at 48 and 96 h after transfection, respectively. This decrease caused a parallel decrease in hTR levels to 16% and 12% of controls after 48 and 96 h, respectively. The rRNA ψ/C ratio was also reduced (72% and 80% of controls for 18S RNA and 86% and 90% of controls for 28S rRNA at 48 and 96 h, respectively). DKC1i = MCF-7 cells transfected with siRNAs specific for dyskerin mRNA sequences; SCR = MCF-7 cells transfected with control scrambled sequences. (b) Telomerase activity assay for (1) heat-inactivated negative control, (2) DKC1-specific siRNA transfected MCF-7 cells, (3) MCF-7 cell transfected with control scrambled RNA sequences, and (4) positive control. Telomerase activity is reduced to 30% by dyskerin reduction 48 h after siRNA transfection. (c) Significant ψ and C peaks in DKC1-specific siRNA transfected MCF-7 cells (left; integrated peak area was 8466 and 53 780 arbitrary units — au — for ψ and C, respectively) and controls (right; integrated peak area was 11 988 and 52 335 au for ψ and C, respectively) 48 h after siRNA.

Discussion

In this study, we investigated for the first time the expression and function of dyskerin in human cancer. First we analysed dyskerin presence and localization by immunohistochemistry on TMAs prepared from a large series of human cancers. Lung, breast, and colon carcinomas were studied, since these are the most frequent tumour types described either in DC patients or in DC animal models [4,6]. Our results demonstrate that dyskerin is, in general, always expressed and properly localized in cancer cells. This is consistent with previous observations indicating that dyskerin function is crucial for the survival of proliferating cells. Indeed, in mouse, DKC1 gene targeting that leads to total loss of function causes early embryonic death [8]. In addition, in Drosophila, loss of function of the minify DKC1 homologue affects fertility, development, or viability, depending on the severity of the mutation [24].

To define more precisely the levels of expression of dyskerin in human cancer, we measured the levels of dyskerin mRNA in a series of breast carcinomas by means of quantitative RT-PCR. The results obtained indicate that the levels of dyskerin expression in breast cancer are highly variable and significantly reduced in a subset of cases. Epidemiological observations in both DC patients [6] and experimental mouse studies [4] show that the partial reduction of dyskerin function can promote neoplastic transformation, thus indicating that dyskerin may act as a tumour suppressor.
Studies in the DKC1 hypomorphic mouse also suggest that the suppressive role of dyskerin is related to its function in rRNA pseudo-uridylation. How defectively pseudo-uridylated ribosome might contribute to neoplastic transformation has not yet been demonstrated. However, it has been hypothesized that the reduction in specific modified uridines could affect regions of the ribosome that are important for translation. This could lead to an impairment of translation of tumour suppressors as well as to the synthesis of aberrant proteins with oncogenic properties [4,25]. We thus investigated the level of rRNA pseudo-uridylation in our breast cancer series. Up to now, ribosome pseudo-uridylation had never been studied in human tumours. This was possibly due to the fact that the most commonly used techniques for this purpose — such as thin-layer chromatography — require a metabolic isotopic labelling step [4,5] and thus cannot be used on patient material. In this study, we describe a reverse-phase HPLC-based method for the quantification of rRNA pseudo-uridylation that can be successfully used starting from unlabelled total RNA. By using this approach, we demonstrated in human breast carcinomas that the dyskerin mRNA expression level is associated with the level of rRNA pseudo-uridylation and suggested that a subset of these tumours is characterized by defective rRNA pseudo-uridylation.

In order to demonstrate that reduced dyskerin expression may influence rRNA pseudo-uridylation, we reduced DKC1 gene expression by specific RNA interference in a breast carcinoma cell line characterized by intermediate expression of dyskerin. We found that a reduction of dyskerin expression actually reduces rRNA pseudo-uridylation. These data, demonstrating that a subset of human breast cancer is characterized by defective rRNA pseudo-uridylation, suggest that the mechanisms that have been proposed to underlie the tumour susceptibility observed in DC patients and DKC1m hypomorphic mice might be also effective in human tumours.

On the other hand, the other major dyskerin function is stabilization of the telomerase complex. Indeed, it has been described that dyskerin binds to the telomerase complex, interacts with hTR, and that hTR levels and telomerase activity are strongly reduced when dyskerin is mutated or underexpressed [3,4]. In most neoplastic and non-neoplastic cell types, hTR is very abundant and its quantity is not related to telomerase activity [26]. However, if the levels of this essential component of telomerase complex are reduced (as occurs in DC), telomerase function can be impaired [27]. In the present study, all the available tissue had been previously used for total RNA extraction. Therefore, it was not possible to assess telomerase activity in the breast carcinoma specimens. To assess the effect of the variable expression of dyskerin on telomerase function, we quantitatively measured hTR levels by real-time RT-PCR. Our results demonstrate that the dyskerin mRNA expression level is strictly associated with hTR levels. Moreover, the dyskerin-dependent regulation of hTR levels has also been demonstrated by selective DKC1 gene silencing in MCF-7 breast cancer cells. These in vitro studies also demonstrate that dyskerin reduction also reduces telomerase activity.

Low levels of telomerase activity have been shown to be associated with good prognosis in breast cancer [28,29]. Therefore, we have hypothesized that dyskerin levels might influence tumour progression by affecting telomerase activity. For this reason, we investigated the relationship between dyskerin mRNA levels and clinical outcome in our series. We found that dyskerin expression is strictly associated with survival: tumours with the lowest dyskerin values are characterized by a more favourable clinical outcome. In order to investigate how dyskerin may influence tumour progression, we compared dyskerin mRNA expression with a series of clinical and bio-pathological parameters that are commonly used to assess the clinical outcome of breast cancer patients. These included age, T and N status, histological grade, ER, PGR, Ki67, p53, p27, RB status, and nucleolar size. Dyskerin expression proved not to be related to any of these variables. Moreover, we performed a multivariate analysis including those parameters that were associated with survival in our series, namely nucleolar size and Ki67. Dyskerin proved to be the only independent factor: low dyskerin levels can therefore be preliminarily proposed as a new index defining a good prognosis in breast tumours.

In conclusion, our results indicate a previously unconsidered significance for dyskerin in human tumour pathology that far exceeds what was already known in the context of the rare syndrome DC. We have demonstrated that dyskerin is always present, in variable amounts, in the nucleolus of human tumours. In a subset of human breast cancer, dyskerin expression was very low and was associated with reduced rRNA pseudo-uridylation. This may be consistent with the tumour suppressive role linked to ribosome function that has been suggested for dyskerin in mouse models [4,25].

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References


