Life Sciences 175 (2017) 31–36

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Dysregulated expression of microRNA-150 in human papillomavirus-induced lesions of K14-HPV16 transgenic mice

Joana M.O. Santos [a,b], Mara Fernandes [a,c,d], Rita Araújo [a,b], Hugo Sousa [a,e], Joana Ribeiro [a,e], Margarida M.S.M. Bastos [f], Paula A. Oliveira [g,h], Diogo Carmo [i], Fátima Casaca [i], Sandra Silva [i], Ana L. Teixeira [a,d], Rui M. Gil da Costa [a,f,g], Rui Medeiros [a,b,d,e,j,*]

[a] Molecular Oncology and Viral Pathology Group, IPO-Porto Research Center (CI-IPOP), Portuguese Institute of Oncology of Porto (IPO-Porto), Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
[b] ICBAS, Abel Salazar Institute for the Biomedical Sciences, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[c] FMUP, Faculty of Medicine, University of Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal
[d] LPCC, Research Department Portuguese League Against Cancer (Liga Portuguesa Contra o Cancro—Núcleo Regional do Norte), Estrada Interior da Circunvalação, no. 6657, 4200-177 Porto, Portugal
[e] Virology Service, Portuguese Institute of Oncology of Porto, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
[f] LEPABE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal
[g] Center for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, UTAD, Quinta de Prados, 5001-911 Vila Real, Portugal
[h] Veterinary Sciences Department, University of Trás-os-Montes and Alto Douro, UTAD, Quinta de Prados, 5001-801 Vila Real, Portugal
[i] Botelho Moniz Análises Clínicas (BMAC), Rua Sarmento de Beires 153, 4250-449 Porto, Portugal
[j] CEBIMED, Faculty of Health Sciences, Fernando Pessoa University, Porto, Portugal

ARTICLE INFO

Article history:
Received 1 February 2017
Received in revised form 7 March 2017
Accepted 11 March 2017
Available online 14 March 2017

Keywords:
HPV16
miRNA-150
K14-HPV16 transgenic mice
Hyperplasia
Dysplasia

ABSTRACT

Aims: High-risk human papillomavirus (HPV) infection is one of the major causes of infection-related cancers worldwide. MicroRNAs (miRNAs) are a family of non-coding RNAs (ncRNAs), whose dysregulated levels may cause an aberrant expression of genes involved in oncogenic pathways and consequently lead to cancer development. This is the case of the miRNA-150 (miR-150), whose expression in HPV-induced lesions remains unclear and the present work aims to clarify it. We employed K14-HPV16 mice, which express the early genes of HPV16 in basal keratinocytes, leading to the development of hyperplastic and dysplastic skin lesions and squamous cell carcinomas, and are a representative model of HPV-induced cancers.
Main methods: In order to evaluate the expression of miR-150 in HPV-induced lesions, we performed qPCR in wild-type mice ($HPV^{-/-}$) and in skin lesions of K14-HPV16 transgenic mice ($HPV^{+/-}$). Matched skin samples were analyzed histologically.
Key findings: 24–26 weeks-old $HPV^{+/-}$ mice showed diffuse epidermal hyperplasia and focal dysplasia in a hyperplastic background (31.8% incidence), but 28–30 weeks-old $HPV^{+/-}$ mice presented higher incidence of dysplasia (100.0%). MiR-150 was upregulated in $HPV^{+/-}$ mice when compared with $HPV^{-/-}$ mice ($p < 0.001$). MiR-150 was also overexpressed in diffuse dysplastic lesions when compared with hyperplastic lesions ($p = 0.005$).
Significance: The present results suggest that miR-150 is overexpressed in HPV-induced lesions in this model and its expression seems to increase with lesion progression, along the process of multi-step carcinogenesis.

1. Introduction

High-risk HPV infection is commonly transmitted by sexual contact and is responsible for approximately 5% of all cancers worldwide [1]. In cervical cancer (CC), which is the second leading cause of cancer mortality in women worldwide, high-risk HPV DNA is present in 99,7% of the cases [2,3].

Current developments, concerning the different players involved in HPV-induced carcinogenesis, suggest that miRNAs play a significant role in the dysregulation of cellular processes [4,5]. MiRNAs are a family of ncRNAs of approximately 22 nucleotides in length that negatively regulate gene expression post-transcriptionally, by translational repression or mRNA degradation, mainly through binding to the 3′-UTR of their target mRNA [6].

* Corresponding author at: Instituto Português de Oncologia do Porto Francisco Gentil, EPE, Grupo de Oncologia Molecular e Patologia Viral – CI-IPOP, Edifício Laboratórios Piso 4, Rua Dr. António Bernardino de Almeida, 4200-072 Porto.
E-mail address: ruimedei@ipoporto.min-saude.pt (R. Medeiros).

http://dx.doi.org/10.1016/j.lfs.2017.03.008
0024-3205/

Several studies have indicated that miRNAs play a role in critical biological events, such as cell proliferation, apoptosis and tumorigenesis [6,7]. In cancer, several miRNAs have shown dysregulated expression in relation to normal tissues and, in some cases, their expression profile have been associated with cancer diagnosis, prognosis and response to treatment [7]. Over the past decade, studies have arisen indicating that miRNAs are possible markers of occurrence and development of HPV-induced cancers and that HPV oncoproteins can interact with miRNAs [5,8–10]. It has also been confirmed that HPV is not able to express its own miRNAs, but it can disturbs host miRNA expression [10]. A study by Martinez et al. showed that the expression of HPV-16 E6 reduced miR-218 expression in HPV16 positive CC [11]. In human foreskin keratinocytes expressing the HPV-16 *E6* oncogene, the E6 oncoprotein induces the upregulation of miR-363 and downregulation of miR-181a, miR-218, miR-29a [12]. Moreover, Greco et al. showed that HPV16 E5 protein alters the expression pattern of miRNAs in HaCaT cells (spontaneously immortalized human keratinocytes) and it seems to favor increased cell proliferation, tumorigenesis and repress epithelial differentiation [9].

In the past few years, miR-150 was first identified by its crucial regulatory role in normal hematopoiesis, but recent studies have shown that the dysregulation of miR-150 is frequently present not only in various types of hematological malignancies but also in a variety of solid tumors [13,14]. Interestingly, depending on the mRNA targeted by miR-150, it can act as oncomiR or tumor suppressor in both malignant hematopoiesis and solid tumors [13,14]. However, the expression profile of this miRNA in HPV-induced lesions remains elusive.

In the present study, we aimed to evaluate the miR-150 expression profile in HPV-induced lesions. For this work, we employed K14-HPV16 transgenic mice, which express the HPV16 early genes in cutaneous and mucosal basal keratinocytes, because these animals develop lesions bearing great similarities to those induced by HPV in human patients, through an identical multistep carcinogenesis process [15–17].

2. Material and methods

2.1. Mice

In order to perform this study, a model of K14-HPV16 transgenic mice on a FVB/n background was used. Creation of K14-HPV16 transgenic mice has been previously reported by Arbeit et al. [17]. These animals express all the early-region genes of HPV16 under the promoter of keratin-14 (K14) with the purpose of directing their expression to the basal keratinocytes of keratinized epithelia. These transgenic mice were kindly donated by Dr. Jeffrey Arbeit and Dr. Douglas Hanahan, through the USA National Cancer Institute Mouse Repository. The animal experiments were approved by the University of Trás-os-Montes and Alto Douro ethics committee (10/2013) and the Portuguese Veterinary Directorate (0421/000/000/2014).

Wild-type mice ($HPV^{-/-}$) and K14-HPV16 transgenic mice ($HPV^{+/-}$) were maintained and bred according with the Portuguese (Portaria 1005/92 dated October the 23rd) and European (EU Directive 2010/63/EU) legislation, under controlled conditions of temperature (23 ± 2 °C), light–dark cycle (12 h light/12 h dark) and relative humidity (50 ± 10%). Health checks were performed daily. Food (4RF21 GLP, Mucedola) and water were provided *ad libitum*.

All mice were previously genotyped by our group through amplification of HPV *E6* and *E2* genes in order to assess HPV integration, as previously described [18,19].

2.2. Experimental design

All samples were obtained from female mice ascribed to four experimental groups (Table 1). Experimental groups were sacrificed at two different timepoints, in order to study different stages of carcinogenesis. Group 1 ($HPV^{-/-}$, $n = 22$) and group 2 ($HPV^{+/-}$, $n = 22$) were maintained up to 24–26 weeks-old; group 3 ($HPV^{-/-}$, $n = 7$) and group 4 ($HPV^{+/-}$, $n = 6$) were maintained up to 28–30 weeks old. Group 4 is less numerous because, HPV16 transgenic animals show high mortality, and only a few survive up to 26–28 weeks old.

Table 1
Experimental groups.

Group	Genotype	Age
1 ($n = 22$)	$HPV^{-/-}$	24–26 weeks
2 ($n = 22$)	$HPV^{+/-}$	24–26 weeks
3 ($n = 7$)	$HPV^{-/-}$	28–30 weeks
4 ($n = 6$)	$HPV^{+/-}$	28--30 weeks

2.3. Samples collection

All mice were sacrificed under ketamine and xylazine anesthesia, by intracardiac puncture and exsanguination, as indicated by the Federation for Laboratory Animal Science Associations. K14-HPV16 mice develop typical HPV-related carcinogenesis in multiple organs; cutaneous lesions (*e.g.* ear and chest skin) are the most frequent and more thoroughly characterized. Chest skin lesions were available and are more suitable for future immunological studies and were chosen for the present work. Chest skin samples from each animal were collected into TripleXtractor reagent (Grisp®), macerated and kept at −80 °C until miRNA isolation. Matched samples were collected for histological analysis.

2.4. Histological analysis

The skin samples collected for histological analysis were fixated in 10% neutral buffered formalin for 48 h. The fixed tissues were then dehydrated through graded alcohols and xylene and embedded in paraffin using an automatic STP 120 processor (Micro, Boise, ID). The paraffin blocks were cut into 2 μm thick sections and stained with haematoxylin and eosin (H&E) for further evaluation under a light microscope. Samples were classified as normal skin, epidermal hyperplasia and epidermal dysplasia. Results were expressed as the percentage of animals showing a specific histological type within the total number of animals in each group.

2.5. MiRNAs isolation

MiRNAs isolation was performed according to Santos et al. [20]. Firstly, it was performed a total RNA extraction using the TripleXtractor reagent (Grisp®), and subsequently the miRNA fraction was isolated with a chloroform solution (Merck®). Then, the miRNAs were purified using the commercial kit GRS microRNA Kit (Grisp®) [21]. MiRNA samples were kept at −80 °C until further use.

2.6. Complementary DNA synthesis

The miRNA samples were then used as templates for complementary DNA (cDNA) synthesis using a TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems®) and sequence-specific stem-loop reverse transcription primers for miR-150 and small nucleolar RNA-202 (snoRNA-202). The amplification conditions were as follows: 30 min at 15 °C, 52 min at 42 °C and finally 10 min at 85 °C. cDNA was further used as template for quantitative real-time PCR (qPCR).

2.7. MiRNA relative quantification

The miRNA expression was measured by qPCR using a StepOne™ qPCR Real-Time PCR device (Applied Biosystems®). For each reaction 1× TaqMan® Fast Advanced Master Mix (Applied Biosystems®) was added with 1× probes (TaqMan® microRNA Expression Assays, miR-