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RESEARCH ARTICLE

Research of the New Biomarkers of Mammary Gland Tumors in Domestic Cats

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ABSTRACT

This article presents the results of the clinical significance study of changes in the activity of adenosine deaminase and the content of polyamines in the blood plasma of cats with mammary gland tumors. In our work, we investigated the activity of the adenosine deaminase gene in the tissues of mammary gland tumors in cats and compared the activity of this gene with normal mammary gland tissue. In the course of this work, we also found out the frequency of occurrence of mammary gland tumors in the population of domestic cats in the Rostov region and evaluated the prognosis of survival after surgical treatment of breast cancer.

The incidence of mammary gland tumors was detected, which amounted to 0.4% of the domestic cats who have applied to veterinary clinics in Rostov region, where the cats with mammary gland neoplasms amounted to approximately 400 individuals for every 100,000 animals. These data reflect the importance of the problem of mammary gland tumors and emphasize the need to study the pathophysiological foundations of carcinogenesis in order to create molecular genetic approaches for early diagnosis of mammary gland tumors.

The results of the studies showed that the level of expression of the adenosine deaminase gene in the tissues of invasive nonspecific carcinoma and in the tissues of fibroepithelial hyperplasia sharply increases compared to the tissue of a healthy mammary gland, 36 times in the tissues of invasive carcinomas and 131 times in the tissues of fibroepithelial hyperplasia. At the same time, there were no significant differences between the activity of adenosine deaminase in the blood plasma of healthy animals and in the blood plasma of animals with malignant neoplasms of the mammary gland. Measurement of the level of polyamines in the blood of animals showed that the content of putrescine in the erythrocytes of the blood of cats with benign and tumor-like neoplasms of the mammary fibroepithelial hyperplasia significantly increased by 5 times compared with the indicators of the control group. In malignant neoplasms of the mammary gland invasive nonspecific carcinoma, the content of putrescine and spermin in the blood significantly exceeded the control values by 6 and 10 times, respectively.

Introduction

Mammary neoplasm is one of the most common oncopathology among small domestic animals. mammary gland tumors i.e. in dogs is in the second place in frequency of occurrence, in cats in third place and differs from other forms of cancer by its aggressive course, malignant nature and poor prognosis. 1,2,3 The foregoing makes researchers look for new biomarkers of mammary gland tumors and develop cost-effective clinical analysis methods to monitor treatment efficacy. 4,5 One of the main features of cancerous tumors is the uncontrolled growth of degenerated cells. Active cell proliferation is accompanied by a change in purine metabolism. Derivatives of purines and their metabolites are involved in the synthesis of protein, DNA, RNA, and in many other biochemical reactions. For example, purine derivatives are necessary for the maturation and proliferation of immunocompetent cells, a violation of the purine mechanism affects the processes of immunoregulation and a decrease in immunity. 6,7,8,9 With the above, it can be assumed that carcinogenesis will be associated with a change in enzyme activity catalyzing the reaction of purine metabolism. Violation of the metabolism of polyamines is considered as one of the molecular mechanisms underlying the pathogenesis of cancer. Polyamines are compounds containing many amino groups. They have a low molecular weight, and are polyfunctional compounds. Polyamines include: spermidine, spermine and diamines - putrescine and cadaverine. In most mammals, and in particular in felines, normally polyamines are present in the blood in extremely low concentrations. The red blood cells take on the function of transporting polyamines; accordingly, in whole blood their level of content will depend on the level of their concentration in the formed elements. Serum and plasma spermine levels are lower than spermidine and putrescine. The amount of spermidine in whole blood predominates. The study of polyamine metabolism in the in-vivo and invitro experiments, as well as clinical and biochemical examinations of sick people with breast cancer, showed that the tumor development process is often accompanied by the accumulation of polyamines in the body. Therefore, their concentration in red blood cells and in blood as a whole is generally considered an indicator of cell proliferation. This indicator has clinical significance mainly when monitoring the dynamics of treatment of cancer patients. Activation of spermidine / spermine acetyltransferase (SSAT) in the tumor tissue causes the formation of several acetyl polyamine derivatives in high concentrations: 1N-acetyl spermidine, 8 N-acetyl spermidine, 12 N-acetyl spermidine, acetyl spermine, etc. The formation of acetyl derivatives of polyamines is associated with reduction of positive charge of molecule and leads to a weakening of their relationship with binding sites with DNA and RNA, which is pathogenetically associated with the progression of the pathological process. Currently, the determination of the content of acetyl derivatives of polyamines in saliva, blood plasma and urine is supposed to be used as a marker of oncology of the intestines, lungs, etc. Cells of tumor tissues usually have a high level of proliferation and, therefore, the synthesis of polyamines is increased. The level of increase in the concentration of polyamines in red blood cells in cancer patients has a direct relationship with the stage of the disease. In turn, this gives the right to consider the quantitative indicator of the polyamine content in erythrocytes as tumor markers, and use the change in this indicator to assess the effectiveness of the therapy. Given the fact that domestic animals live next to humans and are subject to the same carcinogenic factors as humans, the study of tumor markers in domestic animals is an important component for the development of cancer science.

Purpose of the study

The purpose of this study was to learn the clinical significance and to establish the possibility of using as a tumor marker for mammary gland tumors in cats, the determination of adenosine deaminase activity and the content of polyamines in the peripheral blood of sick animals.

Materials and methods

The object of the study was tissue of mammary gland tumors and blood plasma of cats with neoplasms. The blood of healthy animals was used as a control; they were admitted to the hospital for sterilization and were selected on the basis of pairs of analogues. Histological studies were carried out on the basis of the educational laboratory of the Department of Biology and General Pathology, DSTU, the city of Rostov-on-Don. Biochemical studies were carried out on the basis of the biochemical laboratory of the Department of Biochemistry at Rostov State Medical University in Rostov-on-Don. 30 cats with mammary gland neoplasms and 10 healthy animals were examined. Animals were selected for research among patients who came to the clinic for spontaneous tumors in the area of mammary glands. All cats with tumor lesions of the mammary gland were prescribed surgical treatment. At the same time, the patient's age, the degree of operational risk, the size of the neoplasm and the absence of metastases were taken into account (according to the ultrasound and x-ray examination of the chest

and abdominal cavity of the body). First of all, the treatment consisted of a uni and bilateral resection of the mammary glands affected by tumor and regional lymph nodes. The study group included animals with 1 and 2 cancer stage, classification according to TNM. All animals had negative results for viral leukemia in cats FeLV and viral immunodeficiency in cats FIV. The operation was performed in compliance with the principles of ablastic and antiblastic. The research protocol was in accordance with the guidelines of the Ethics Committee for Work with Animals of the Federal State Budget Educational Institution of Higher Education of the Rostov State Medical University on July 26, 2018.Operational material was photographed, measured, described and fixed in 10% buffered formalin. After fixation, histological preparations (paraffin blocks and glasses) were cut and manufactured according to the standard procedure. The diagnosis of invasive non-specific breast carcinoma and hyperplastic changes was made according to the nomenclature and criteria of histological classification of mammary gland tumors of the World Health Organization 2012, indicating the degree of differentiation.

After the histological diagnosis, tissue was taken from the corresponding tumor area for molecular studies. Total RNA was isolated from mammary gland tumors tissue samples and healthy mammary gland tissue by guanidintriocyte phenol chloroform extraction.10 The mRNA levels of the B2M, GAPDH, and ADA genes were determined by real-time PCR in the presence of the SybrGreen 1. The amount of PCR product cDNA of these genes, which we obtained as a result of the reverse transcription reaction, was measured in each PCR cycle using a fluorescent dye. Primers used for analisis presented in table 1. After that, the activity of the enzyme Adenosine deaminase in blood plasma was determined by the Kalear method.11 The content of polyamines was determined in red blood cells of venous blood by the method of high-performance liquid chromatography on a column Lichrosphere RP18, pre-converting them to benzene derivatives for identification in UV at 229 nm.

Table 1. Primers used for enzymatic amplification of genes (B2M, GAPDH, and ADA) of the studied objects

Forward /reverse primer name	Primer structure
B2M forward	TTTGTGGTCTTGGTCCTGCTCG
B2M reverse	TTCTCTGCTGGGTGACGGGA
GAPDH forward	AGTATGATTCCACCCACGGCA
GAPDH reverse	GATCTCGCTCCTGGAAGATGGT
ADA forward	TTCGACAAGCCCAAAGTGGAG
ADA reverse	AACGCACCTCCACATACACC

Results

Analysis of 73247 medical histories of cats living in Rostov-on-Don and Rostov region showed that the number of complaints about mammary gland tumors amounted to 321 animals, about 0.4 % of patients. Accordingly, the incidence of mammary gland tumors was approximately 400 individuals for every 100 000 cats. 30 domestic cats who applied to the veterinary clinic for spontaneous tumors in the area of milk bags were operated on from our team. 3 years later we finded out that cats aged 8 years and older should be included in the risk group for the development of mammary gland tumors. The average age of animals at which they develop breast cancer is 12.9 years. 90% of the animals in the study group were not sterilized. In 80% of the animals in the anamnesis, the owners indicated the use of hormonal drugs in the form of drops or injections of proligesnone. The survival rate after surgery to remove a breast tumor among animals with diagnosed fibroadenomatous hyperplasia was 100%, the animals felt well for 3 years of the study. Two cats had offspring, after which the animals were sterilized. In the group of animals with malignant neoplasms, 11% died within 3 weeks due to post-surgical complications or even was euthanized at the request of the owners. 14% lived after surgery for more than 2 years and died of natural causes. 18% of the animals had a relapse, and they died within 3-8 months after surgery. Eeveryone else was fine 3 years after surgery. Thus, the survival rate for 3 years of animals with stage 1-2 mammary gland tumors after surgical treatment was 70%.

The analysis of the tumor tissue that we received as a result of the treatment of our 30 animals showed that in 3 animals the tumor was fibroepithelial hyperplasia. These were young animals, aged from 8 months to 2 years, all had a history of taking hormonal drugs based on exogenous progestins. One animal was diagnosed with pericanalicular fibroadenoma. This animal has already had an operation to remove a breast neoplasm, which was fibroadenomatous hyperplasia, but the owner instead of the

recommended sterilization continued the use of progestin drugs. 26 animals were diagnosed with breast cancer of varying degrees of malignancy. The age of the animals ranged from 8 to 19 years. In the vast majority of cases, we registered ductal cancer. Thus, malignant neoplasms accounted for 87% of all the animals we operated on. The studied animals were divided into groups based on the malignancy of the tumor and the degree of differentiation. The control group included clinically healthy animals who were admitted to the clinic for sterilization. The group of animals with benign and tumor-like neoplasms consisted of animals with fibroepithelial hyperplasia and fibroadenoma of the mammary gland. The groups of animals with malignant neoplasms consisted of animals with invasive nonspecific carcinoma of low, moderate and high degree of differentiation.

Next, we started molecular studies of tumor tissue. We used DNA primers developed by us for the ADA gene encoding the enzyme adenosine deaminase Table 1. Simulated mRNA-specific DNA primers were tested in three different concentrations in real-time PCR mode (125, 250 and 500 Nm) of direct and reverse primers. All samples contained a final DNA concentration of 0.2 NG/ml. The melting curve was analyzed at temperatures from 60 °C to 99 ° C. PCR reactions were linear in the range of eight log10 of the synthetic template, and gave one peak in the analysis of the melting curve. Amplification of mRNA isolated from biological samples yielded the same amplification curves as for synthetic templates. Analysis of the melting curve showed the presence of only one amplicon. In addition, when using biological samples, a good correlation was observed between Ct and template concentration in four dilutions, which confirmed the high specificity and sensitivity of the developed primers. When selecting reference genes, the B2M gene showed high expression in all tissue samples and had a low coefficient of variation, unlike the GAPDH gene. In addition, according to the literature 12, usually, when studying the expression of genes associated with the development of mammary gland tumors, the B2M gene is used as a reference. Therefore, the expression of ADA genes was calculated in relation to the expression of the B2M gene. The level of expression of the ADA gene increased significantly in the tissues of mammary gland neoplasms compared to healthy mammary gland. The results of the study are presented in Tables 2-3.

Table 2 -ΔCt indices for the ADA gene in groups of animals with neoplasms and in the control group

Groups ADA	Control n =10	Invasive non-specific carcinoma n =26	Fibroepithelial hyperplasia/ fibroadenoma
ΔCt (M±m)	0,4±0,07	5,57±0,31	7,44±0,59

Table 3 - Indicators of the level of expression of ADA in tumor tissues in relation to indicators in healthy mammary gland tissues

Groups	Δ ΔCt	2 - Δ ΔCt	U-Mann-Whitney criterion(p)
Invasive non-specific carcinoma	5,17	36	p<0,001
Fibroepithelial hyperplasia/ fibroadenoma	7,04	131	p<0,001

Given the increased expression of the gene encoding the enzyme adenosine deaminase, we expected to see an increase in the activity of this enzyme in the peripheral blood of animals with mammary gland tumors. The results of the study of ADA activity in the blood of cats are presented in table 4.

Table 4. ADA activity in the blood plasma of healthy cats and cats with neoplasms (M±m, in nmol / sec / ml of blood plasma).

Groups	ADA Enzyme Activity
Control n=10	67±5
All neoplasms n=30	54±15 (t =1,00, p = 0,212)
Fibroepithelial hyperplasia/ fibroadenoma n=4	45±2 (t = 5,00, p = 0,007)
Highly differentiated invasive non-specific carcinoma n=10	50±10 (t =1,271, p = 0,261)
Moderately differentiated invasive non-specific carcinoma n=9	47±2 (t =1,33, p = 0,22)
Low differentiated invasive non-specific carcinoma n=7	68±2 (t =0,278, p = 0,79)

According to the results obtained, there were no significant differences between the activity of ADA in the blood plasma of healthy animals and in the blood plasma of animals with malignant neoplasms of the mammary gland. Depending on the degree of tumor differentiation, no significant differences in enzyme activity were also found. Since the analysis of the activity of ADA showed that measuring the activity of this enzyme in the blood of animals with mammary gland tumors cannot be used as biomarkers of mammary gland tumors, we decided to analyze the content of polyamines in the blood of the same animals.

We obtained indicators of the level of polyamines in the blood of healthy cats: putrescine - 0.04 mcg/ml, spermidine - 5.94 mcg/ml and spermine - 1.63 mcg/ml. In benign and tumor-like neoplasms of the mammary gland, the content of putrescine in the blood of cats statistically significantly increased by 5 times ($p<0.0001$) compared with the indicators of the control group. In malignant neoplasms of the mammary gland (invasive nonspecific carcinoma), the content of putrescine and spermin in the blood significantly exceeded the control values by 6 ($p<0.0001$) and 10 times ($p<0.02$), respectively. At the same time, the content of spermidine in the blood of cats, on the contrary, decreased by 8 times ($p <0.02$) compared with the control. The decrease in the content of spermidine, apparently, is associated with the formation of various acetyl derivatives table 3.

Table 5 - Polyamine content in the blood of cats with mammary gland neoplasms (M ± m, in mcg/ml of blood)

Polyamines, µg/ml			
Groups	Putrescin	Spermidine	Spermine
Clinical norm	0,4±0,01	5,94±1,13	1,63±0,30
Benign neoplasms	2,15±0,22 **	4,00±0,40	2,68±1,00
Malignant neoplasms	2,45±0,67*	0,76±0,14*	15,56 ±3,76*

* - p1<0.02 - significance of differences in values in patients compared with the norm.
** - p1<0.0001 - significance of differences in values in patients compared with the norm.

Conclusions

The survival rate for 3 years of animals with stage 1-2 mammary gland tumors after surgical treatment was 70%. They once again confirm the effectiveness of the treatment of breast neoplasms in the early stages. Therefore, it is necessary to find markers that will appear in the blood at the early stages of the occurrence of neoplasms, which will allow for early diagnosis.

According to the literature, adenosine deaminase performs hydrolytic deamination of adenosine to inosine, and in rapidly proliferating tissues, for example, in lymphocytes and intestinal cells, the activity of this enzyme usually changes.13 The degeneration of healthy mammary gland cells into tumor cells is always accompanied by uncontrolled tissue growth, respectively, the expression of the ADA gene should change. To confirm the correctness of the conclusions made, a pairwise comparison of the average ΔCt values of the studied mRNAs of the control group with the groups of invasive nonspecific carcinoma and fibroepithelial hyperplasia was carried out using the Mann-Whitney U-test, the data of which are also given in Tables 6-7. The results obtained confirm that the level of expression of the ADA gene in the tissue of tumors of invasive nonspecific carcinoma and in the tissue of fibroepithelial hyperplasia statistically significantly differ from the corresponding indicator in the control group ($p<0.001$). The level of expression of the ADA gene in the tissues of tumors of invasive nonspecific carcinoma and in the tissues of fibroepithelial hyperplasia increases sharply compared to the tissue of a healthy mammary gland (36 times in the tissues of invasive carcinomas and 131 times in the tissues of fibroepithelial hyperplasia). The results of the study confirm the involvement of the ADA gene in the pathogenesis of oncological diseases. Considering the above, the detection of changes in the number of certain mRNAs can be used to understand both the pathophysiological foundations of carcinogenesis and to search for molecular genetic approaches to its early diagnosis.

Were no significant differences between the activity of ADA in the blood plasma of healthy animals and in the blood plasma of animals with malignant neoplasms of the mammary gland. Apparently, this is due to the fact that the examined animals were operated on at stage 1-2 of breast cancer and the pathological processes were not significant enough to affect the metabolism of the body as a whole. Another possible explanation for the lack of an increase in the activity of ADA in the blood of sick animals may be the fact that, basically, ADA in the blood is contained in

lymphocytes. In immunodeficient conditions, there is usually an insufficiency of ADA, therefore, a drop in the production of ADA by lymphocytes can compensate for an increase in the activity of ADA synthesized by tumor tissue.

In most mammals and, in particular, in representatives of cats, polyamines are normally present in the blood in extremely low concentrations. The function of polyamine transport on erythrocytes take over, respectively, in whole blood, their level of content will depend on the level of their concentration in the shaped elements. The content of spermin in serum and blood plasma is lower than spermidine and putrescine. The amount of spermidine in whole blood prevails. 14 The study of polyamine metabolism in experiments in vivo and in vitro, as well as clinical and biochemical examinations of patients with breast cancer showed that the process of tumor development is often accompanied by the accumulation of polyamines in the body. Therefore, their concentration in red blood cells and in the blood as a whole is considered to be an indicator tissue necrosis. This indicator has clinical significance mainly when observing the dynamics of treatment of people with oncological diseases. Tumor tissues usually have a high level of necrosis and, consequently, in this regard, the synthesis of polyamines increases. The level of increase in the concentration of polyamines in erythrocytes in cancer patients has a direct relationship with the stage of the disease. In turn, this gives the right to consider the quantitative indicator of the content of polyamines in red blood cells as cancer markers, and use the change in this indicator for evaluation of the effectiveness of the therapy.15 A sharp increase in the level of polyamines in body fluids is observed with the growth of various neoplasms. The exchange of polyamines is actively manifested in the proliferation and cellular differentiation of tissues; therefore, it is conducted active development of targeted anticancer drugs on the exchange of polyamines.16 Measurement of the level of polyamines in biological fluids can be used to assess the effectiveness of the treatment. Based on the work done, we can draw the following conclusions:

1. The incidence of mammary gland neoplasms in cats of the Rostov region is 400 animals over 100,000 cats. So, 0.44% of the cat population of the Rostov region have mammary gland neoplasms.
2. 87% of mammary gland tumors in cats are malignant.
3. The level of expression of the ADA gene in the tissues of malignant and benign tumors is statistically significantly higher than in the tissues of a healthy mammary gland (36 times in the tissues of invasive carcinomas and 131 times in the tissues of fibroepithelial hyperplasia).
4. The level of adenosine deaminase activity in the blood does not change. The activity of AD in the group of healthy animals is 67 ± 5 nmol / sec / ml of blood plasma, in the group of animals with mammary gland tumors 54± 15 nmol / sec / ml of blood plasma.
5. In the blood of cats with mammary gland tumors, the level of polyamines significantly increases. The content of polyamines in the blood of healthy animals is: putrescine 0.04± 0.01 mcg/ml, spermidine 5.94±1.13 mcg/ml, spermine 1.63±0.30 mcg/ml. The content of polyamines in the blood of animals with mammary gland tumors is: putrescine 2.45 ± 0.67 mcg/ml, spermidine 0.76± 0.14 mcg/ml, spermine 15.56 ±3.76 mcg/ml.

References

1. Overley-Adamson B. Baez J. Feline Mammary Carcinoma. Feline internal medicine. 2016(7); 578-584. doi.org/10.1016/B978-0-323-22652-3.00059-1
2. Garden O. Volk S. Masson N. Perry J. Companion animals in comparative oncology: One Medicine in action. The Veterinary Journal. 2018(240);6-13. doi.org/10.1016/j.tvjl.2018.08.008
3. Withrow J. Vail D. Rodney L. Withrow & MacEwen's. Small Animal Clinical Oncology. 2013 (5);1-29. doi.org/10.1016/B978-1-4377-2362-5.00001-3 Matos A. Santos A. A modern look at the molecular-biological mechanisms of breast tumours in dogs. The Veterinary Journal. 2015 (205);136-143. doi.org/10.1016/j.tvjl.2015.02.004
4. Hughes K. Dobson J. Prognostic histopathological and molecular markers in feline mammary neoplasia. The Veterinary Journal. 2012(194);19-26. doi.org/10.1016/j.tvjl.2012.05.008
5. Matosab A. Baptistaac C. Gärtnerad M, Ruttemane G. Prognostic studies of canine and feline mammary tumours: the need for standardized procedures. The Veterinary Journal. 2012(193);24-31. doi.org/10.1016/j.tvjl.2011.12.019
6. Abbed K. El-Said H. Hamdia Y. Askar Basma Y.El. Kady A. Performance of serum adenosine deaminase in measuring disease activity in rheumatoid arthritis patients. The Egyptian Rheumatologist. 2018;05. 005. doi.org/10.1016/j.ejr.2018.05.005
7. Castilhos L. Adefegha S. Doleski P. Bertoldo T. Eduardo C. Moritz J. Casal E. Altered E-NTPDase/E-ADA activities and CD39 expression in platelets of sickle cell anemia patients. Journal of Applied Biomedicine. 2018(16); 208-213 doi.org/10.1016/j.jab.2017.12.004
8. Namiot Z. Kemona A. Stasiewicz J, Marcinkiewicz M. Adenosine deaminase activity in gastric cancer. Cancer Letters. 1994 ; 95-98 (1994) doi.org/10.1016/0304-3835(94)90151-1
9. Battist V. Maders L. Bagatini M., Battisti E., Bellé L. Santos K. Ectonucleotide pyrophosphatase/phosphodiesterase (E-NPP) and adenosine deaminase (ADA) activities in prostate cancer patients: influence of Gleason score, treatment and bone metastasis. Biomedicine & Pharmacotherapy. 2013; 203- 208. doi.org/10.1016/j.biopha.2012.12.004
10. Chomczynski P. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. P. Chomczynski, N. Sacchi . Biochemistry. 1987 ; 2687-97. doi.org/10.1006/abio.1987.9999
11. Berezin I.V. Practical course of chemical and enzymatic kinetics. I.V.Berezin. A.A. Klesov. Moscow. 1976 ; 325.
12. Penning, L. C. A validation of 10 feline reference genes for gene expression measurements in snap-frozen tissues. L. Penning, H. Vrieling, B. Brinkhof. Veterinary Immunology and Immunopathology. 2007; 212–222. doi: 10.1016/j.vetimm.2007.08.006
13. Vy Hong-Diep. Kim. Neutropenia among patients with adenosine deaminase deficiency. Vy Hong-Diep Kim. Anne Pham-Huy. Eyal Grunebaum. Journal of Allergy and Clinical Immunology. 2019 (143): 403-405. doi: 10.1016/j.jaci.2018.04.029
14. Igarashi K. The functional role of polyamines in eukaryotic cells. K. Igarashi, K. Kashiwagi. Int J Biochem Cell Biol.2018.107; 104-115. doi: 10.1016/j.biocel.2018.12.012
15. Cervelli M. Polyamines metabolism and breast cancer: state of the art and perspectives. M. Cervelli, S. Pietropaoli, F. Signore, R. Amendola, P. Mariottini. Breast Cancer Res Treat.2014. 148; 232-248. doi: 10.1007/s10549-014-3156-7
16. Nowotarski S.L. Woster P.M. Casero R.A.Jr. Polyamines and cancer: implications for chemotherapy and chemoprevention / S. L. Nowotarski, P. Woster, R. Casero // Expert Rev Mol Med. 2013; 15-23. doi: 10.1017/erm.2013.3