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Study of biomarkers for characterization of mammary tumours in Wister rats induced by N-methyl nitrosourea

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Abstract

The present study was conducted with the objective to study the expression of molecular markers in N-methyl nitrosourea (NMU) induced mammary tumours in female wister rats. The molecular markers for proliferation, angiogenesis and apoptosis at mRNA level studied with the help of RT-PCR. Mammary tumour was induced by injecting NMU intraperitoneally into female virgin Wister rats @ 200 mg/kg body weight at 50, 80 and 110 days of age. RT-PCR reaction was carried out to verify the mRNA expression of the PCNA, p53, VEGF and caspase-3 genes related to cancer progression and regression in mammary tumour. The RT-PCR condition was carried out using PCR master mix (MBI Fermentas, USA) following standard thermal profile and cycling conditions. Gel electrophoresis showed specific product sizes of 198 bp, 289 bp, 240 bp and 178 bp for PCNA, p53 and caspase-3 respectively. The expression of VEGF three most common isoforms in rat VEGF₁₂₀, VEGF₁₆₄ and VEGF₁₈₈ showed specific product size of 635bp, 563bp and 431bp respectively. However, no significant differences were found for expression of these genes among the different groups.

Keywords: Mammary, tumour, rats, RT-PCR, biomarker

Introduction

Cancer remains a challenge to mankind due to its fatality and incurability. It is one of the leading health concerns for humans accounting one in eight deaths worldwide (Garcia *et al.*, 2007). Cancer is the second leading cause of death across the globe (Bray *et al.*, 2018) [3]. As per the GLOBOCAN estimates, in the year 2020, the International Agency for Research on Cancer (IARC) recorded 19.3 million new cases of cancer and approximately 10.0 million cancer-related deaths worldwide. Breast cancer is one of the most prevalent types and most rated common malignancy in women and female dogs (Ghoncheh *et al.*, 2016; Kaszak *et al.*, 2018) [4, 5]. Due to ethical issue and scarcity of human tissue sampling, various laboratory animals are used as human breast cancer models in recent years (Abdelmegeed and Mohammed, 2018). The suffering of cancer affected animals is yet to be ameliorated by safe, efficacious and economical cancer therapy. Before treatment it is utmost important to understand the mechanism of cancer development. The association of chemicals as a causative agent of cancer leads to the development of *in vivo* and *in vitro* experimental models and other technical advances facilitating a molecular approach to the problems in oncology. Mammary carcinogenesis can be induced experimentally in laboratory animals by a variety of agents such as hormones, viruses, irradiations, chemicals etc. (Russo and Russo, 2000) [7]. Although histopathology is primarily used for diagnosis and differentiation of benign from malignant tumours, detection of expression pattern of oncogenes and other related genes/proteins as molecular markers such as Ki67, proliferating cell nuclear antigen (PCNA), estrogen receptor (ER), c-erbB2, progesterone receptor (PR), apoptotic genes like Bcl-2, p53 and Vascular endothelial growth factor (VEGF) are being increasingly used to aid in diagnosis, prognosis and assessment of therapeutic response.

Materials and Methods

The present study was conducted with the objective to study expression of molecular markers in N-methyl nitrosourea (NMU) induced mammary tumours in female wister rats. The molecular markers for proliferation, differentiation, angiogenesis and apoptosis at mRNA level studied with the help of RT-PCR. Mammary tumour was induced by injecting NMU intraperitoneally into female virgin wister rats @ 200 mg/kg body weight at 50, 80 and 110 days of age. The animals were sacrificed at end of 20 weeks experimental periods. RT-PCR reaction was carried out to verify the mRNA expression of the PCNA, p53, VEGF and caspase-3 genes related to cancer progression and regression in

mammary tissue exposed to NMU.

RT-PCR reaction was carried out to verify the mRNA expression of the PCNA, p53, VEGF and caspase-3 genes related to cancer progression and regression in mammary tissue exposed to NMU with or without herbal treatment as following standard protocol.

Extraction of total RNA

The total RNA was extracted from tumour tissue and normal tissue mammary gland with TRI reagent (T9424, Sigma Aldrich, USA) as per the manufacturer instructions. The thermal profile for various reactions were as follows.

Sl. No.	Genes	Steps					No. of cycles
		Initial Denaturation	Denaturation	Annealing	Elongation	Final Elongation	
1	PCNA	95 °C-3 min	95 °C-45 sec	52 °C-45sec	72 °C-45sec	72 °C -5 min	35
2	p53	94 °C-3 min	94 °C-45 sec	52 °C-30 sec	72 °C-30 sec	72 °C-5min	30
3	VEGF	94 °C-3 min	94 °C-45 sec	56 °C-30 sec	72 °C-30 sec	72 °C-5 min	35
4	Caspase-3	94 °C-3 min	94 °C-30sec	54 °C-30 sec	72 °C-30 sec	72 °C-5 min	35
5	GAPDH	95 °C-4 min	94 °C-30 sec	56 °C-30 sec	72 °C-30 sec	72 °C-5 min	30

Results and Discussion

A total of 39 tissues, 30 from tumours tissue from different treatment groups and 9 normal tissue from control group animals were subjected to reverse transcription polymerase chain reaction (RT-PCR) to study expression of molecular markers for cell proliferation, differentiation, angiogenesis and apoptosis *viz.* PCNA, p53 and caspase-3 at mRNA level in NMU induced rat mammary tumours. Gel electrophoresis showed specific product sizes of 198 bp, 240 bp and 178 bp for PCNA, p53 and caspase-3 respectively. The expression of VEGF three most common isoforms in rat VEGF₁₂₀, VEGF₁₆₄ and VEGF₁₈₈ showed specific product size of 635bp, 563bp and 431bp respectively.

The detection of mRNA transcripts for specific epithelial markers by using reverse transcriptase polymerase chain reaction (RT-PCR) and more recently, the quantitative real-time RT-PCR (QPCR) seems to have higher diagnostic

sensitivity (Ring *et al* 2005) [8]. However, Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen and a key regulator of angiogenesis in a variety of physiologic and pathologic processes including cancerous process and produces various VEGF iso-forms differing in length. In humans, five different VEGF mRNAs have been detected encoding the isoforms VEGF₁₂₁, VEGF₁₄₅, VEGF₁₆₅, VEGF₁₈₉, and VEGF₂₀₆ (Neufeld *et al.* 1999) [6]. Chengshi *et al.* (2002) [2] detected splice variants of VEGF in Copenhen rat during mammary carcinogenesis We have detected VEGF splice variants of VEGF₁₈₈, VEGF₁₆₄ and VEGF₁₂₀ of 635, 564 and 432 bp, respectively using RT-PCR assay. In the present study we have detected 3 isoforms of VEGF, VEGF₁₂₀, VEGF₁₆₄ and VEGF₁₈₈ showed specific product size of 635bp, 563bp and 431bp respectively. The findings in the present study are in corroboration with earlier reports.

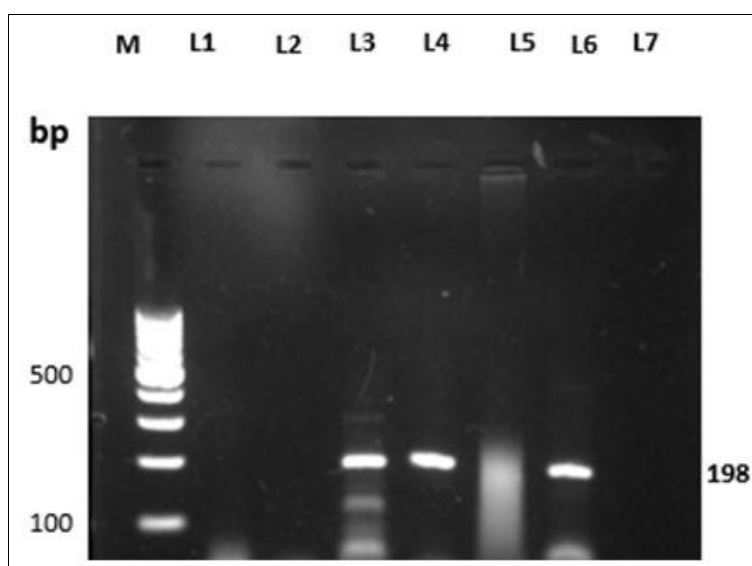


Fig 1: PCR products in ethidium bromide stained 1% TBE 1% TBE gel electrophoresis M-100 bp marker L1-L7 PCNA-198 bp

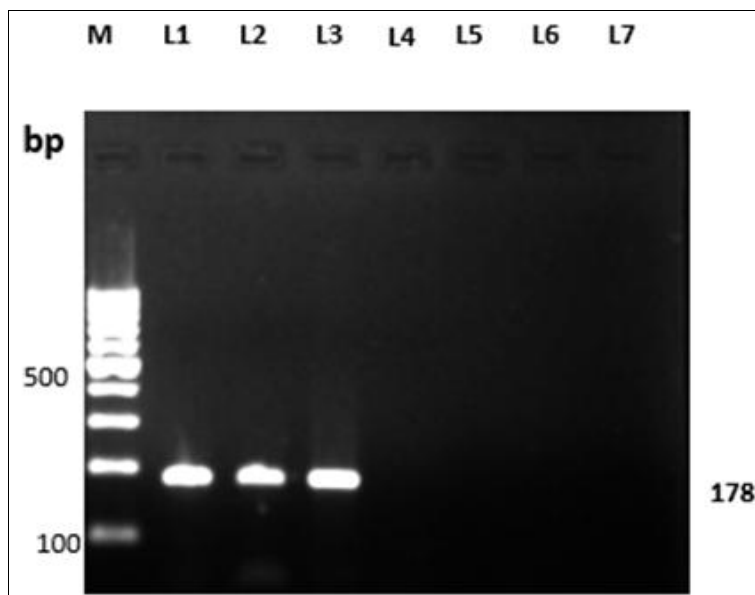


Fig 2: PCR products in ethidium bromide stained gel electrophoresis M-100 bp marker, L1-L7 caspase-3;178 bp

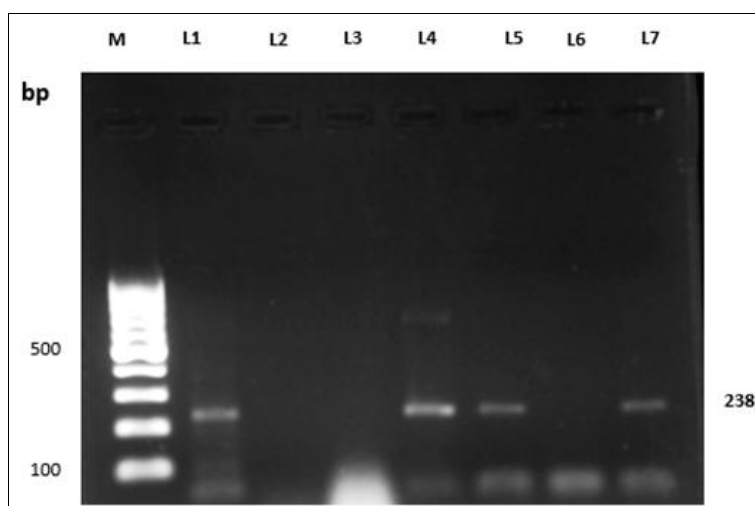


Fig 3: PCR products in ethidium bromide stained 1% TBE gel electrophoresis M-100 bp marker, L1-L7 p53-238 bp

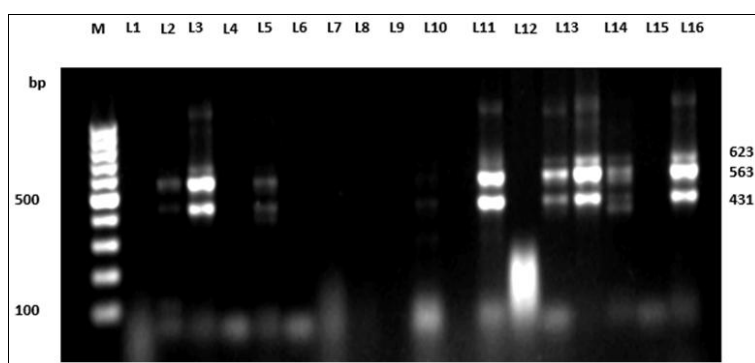


Fig 4: PCR products in ethidium bromide stained 1% TBE gel electrophoresis M-100 bp marker, L1-L16 VEGF-431bp, 563 bp& 623 bp

Conclusion

The present study provide better understanding of the molecular alterations that occur during carcinogenesis by evaluating the biomarkers through RT-PCR is and suggest importance of molecular markers for proliferation, angiogenesis and apoptosis at mRNA level for progression and regression during the process of tumourogenesis.

Conflict of Interest

Not available.

Financial Support

Not available.

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