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ORIGINAL ARTICLE

# The importance of habitat in the tumor-associated *Pten*, *Mtor*, and *Akt* gene expressions and chromosomal aberrations for wild rats

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## Abstract

The value of PTEN in tumors has long been appreciated including colorectal cancer (CRC). It is a negative regulator of the PI3K/AKT/mTOR signaling pathway that is known to be actuated in human cancers. However, PTEN-regulated modulation of the main pathway components in different rat ecosystems is unknown. The results demonstrated the negative transcriptional correlation between *Pten* and *Mtor* and *Akt* genes in rats living in an urban or rural habitat. The downregulation of *Pten* in colon epithelium led to the upregulation of *Mtor* and *Akt* genes, possessing risk in terms of CRC for urban rats. However, for rural rats this condition was vice versa, emphasizing that rural habitat can be protective in terms of CRC. The study is valuable for the identification of the link between cancer susceptibility and urban/rural environments. Furthermore, the cytotoxic effects of urbanization in rat populations were determined by detecting chromosomal aberrations. Due to the expansion of industrial and agricultural activities of the growing human population, the rapid vanishing of natural habitats is inevitable. Being also “messengers” of environmental pollution, the disclosure of health-affecting cellular processes in different habitats of rats living in close proximities to humans will contribute to environmental policies and human wellness.

**Keywords** Cancer · Colon epithelium · Chromosome aberrations · Habitat differences · *Rattus rattus* · PTEN

## Introduction

The genus *Rattus* has followed human migration throughout history, dispersing from Asia to Europe, and then to the rest of the world, thanks to long-distance journeys like the Silk Route and trans-Atlantic traffic (Puckett et al. 2016). Today, rats can be found nearly everywhere on the planet because of their physical versatility and omnivorous nutrition. Their prevalence is related to humans and their capability to colonize a wide range of man-made ecosystems (Feng and Himsworth, 2014). Rapid urbanization, poverty, slums with insufficient infrastructure, the abundance of food, and inappropriate garbage management, allow the rats to be close to humans in urban environments. Therefore, the spread of carried human pathogens is also increasing (Benacer et al. 2016). The habitats of rats in cities are unevenly spread, and each rat’s natural habitat is comparatively small relative to animals in less urban locations. Commensalism has many regional roots, and black rats have a complicated dissemination pattern. Rats, like most mammals, exhibit female philopatry and male dispersal (Gardner-Santana et al. 2009) and they select their territory based on insulation, nutrition, and water supply (Modlinska and Pisula, 2020). Rats are curious and excited to discover new places, but they suffer from neophobia. They also significantly decrease their calorie intake upon being subjected to a new recipe. The initial fear of the new food is accompanied by incremental testing (Modlinska and Pisula, 2020). According to scholars, city rats are one of the most common but understudied wildlife species in urban areas (Parsons et al. 2017). Considering the close proximity of humans and rats, our understanding of rat populations in urban locations is yet limited (Combs et al.

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2018). Furthermore, little is understood about how the ecosystem affects these populations on a regional level (Zeppelini et al. 2021).

The development of urban ecosystems reduces the diversity and richness of native species but creates new anthropic habitats that allow rodent populations to live (Cavia et al. 2009). Among rodents, the adaptability of *Rattus rattus* (Linnaeus, 1758), which allows them to live successfully in a wide variety of environments, is quite remarkable (Goedert et al. 2020). As a result of the rat's adaptation to changing environmental conditions, some differences in body size, skull, and skeletal features have been detected (Pergams et al. 2015). It has been shown that there is a significant difference in tooth wear depending on diet in rats in human settlements compared to rats in natural environments with similar abiotic conditions (Winkler et al. 2016). By comparing the dominant vegetation in the region, *R. rattus* stomach nematodes were previously examined, and the study stated that the only strong and consistent factor associated with the average abundance of nematodes in rats is habitat (Lafferty et al. 2010). In a recent metagenomic study, the effects of nutritional and lifestyle changes in different habitats were explained by comparing the gut microbiota of *R. rattus* living in urban and rural habitats at the taxonomic level (Gurbanov et al. 2022).

As a driver of urbanization, climate change and pollution fundamentally alter both biotic and abiotic ecosystem characteristics (Grimm et al. 2008). When urban habitats for wild animals are compared to non-urban habitats; it is defined by some characteristics, such as higher temperatures, reduced humidity, habitat degradation, pollution (chemicals, dust, garbage, etc.), human density, excess human food, and the presence of domestic animals (Adams et al. 2005). Chemical pollution, which is one of the important environmental stress factors that urban organisms are exposed to, is among the constant elements of urban environments. Especially with modern urbanization and industrialization, heavy metal contamination has become a primary concern for today's society (Srivastava et al. 2017). Some rodent species are used as bioindicators of regional pollution in ecotoxicological studies (Kumar Sharma et al. 2007). In these studies, besides histopathological and biochemical changes, the biological effects of environmental pollution on individuals and populations were evaluated with cytogenetic biomarkers such as chromosome aberrations, comet assay in peripheral blood cells, micronucleus assessment in peripheral erythrocytes, bone marrow nucleus test, and abnormal sperm morphology (Alimba et al. 2021). However, reports on the effect of regional factors on rodents at the specific gene level have been limited. Chromosomal aberrations have been recognized as a very sensitive endpoint to detecting genotoxic effects induced by heavy metals and toxic elements (Topashka-Ancheva et al. 2003). It is also a biomarker in human biomonitoring studies that indicates an increased risk of environmental carcinogens (Sram et al. 2007). It has been stated that there is a relationship between the frequency of chromosomal aberration and the risk of gastric and possibly colon and rectal cancer (Boffetta et al. 2007). In wildlife, biological systems are affected at nearly all levels, from molecules to ecosystems, as a result of chronic or acute exposure of rodents to toxicity and pollution, often through the food chain (Sánchez-Chardi and Nadal, 2007). Besides heavy metals, commonly used rodenticides are other important toxic chemical pollutants to which wild rats are exposed in urban environments (Lattard and Benoit, 2019). Bromethalin found in some rodenticides is considered a carcinogenic substance. However, there is no evidence of carcinogenesis from long-term exposure to bromethalin in animal and human studies (Mercer et al. 2022).

The extent to which animals can cope with urban environments affects all aspects of their fitness and survival (Gauvin et al. 2016). Along with the reducing effects of species richness, the morphological, behavioral, and physiological consequences of urbanization in natural populations including mammals, birds, reptiles, amphibians, and plants have been the subject of many studies (McKinney, 2008; Alberti et al. 2017a; Putman and Tippie, 2020). Although research on the ecological effects of urbanization has increased steadily, only recent studies have been focused on the determination of the molecular basis of environmentally induced phenotypic variations. These studies expand our understanding of how ecological processes and the environment shape the phenotype (Watson et al. 2017). Still little is known about the health, life history strategies, and causes of death of wild animals living in cities, or the mechanisms by which wild animals adapt to urban conditions (Sepp et al. 2019).

Urbanization and industrialization, as well as environmental and nutritional changes, have brought important health problems to humans (Kuddus et al. 2020). In the last two decades, the number of colorectal cancer (CRC) patients has been increasing rapidly, especially in urban areas (Wei et al. 2022). Loss of PTEN (phosphatase and tensin homologue deleted on chromosome 10) has been studied as a valid biomarker in the tumorigenesis and prognosis of CRC. The potential of therapeutics targeting PTEN deficiency in the treatment of CRC has also been highlighted (Sadat et al. 2021). PTEN is a phosphatase with both protein and lipid phosphatase activities (Cai et al. 2009). While PTEN activation prevents cell growth and induces apoptosis in intestinal carcinoma cells, its downregulation facilitates cell proliferation, longevity, and epithelial-mesenchymal transformation (Bowen et al. 2009; Byun et al. 2011). PTEN deletion in the intestine induced pAKT expression and the likelihood of tumor development in mice. These results support PTEN's function as a true tumor-suppressor gene in intestinal cancers (Byun et al. 2011). The PI3K/AKT/mTOR signaling pathway is fundamentally activated in CRC and contributes to tumorigenesis by activating downstream targets such as protein kinase B (AKT) and mammalian target of rapamycin (mTOR) (Zhang and Yu, 2010; Byun et al. 2011). Many human disorders are caused by abnormalities of the serine/threonine kinase, AKT. It is transiently triggered in tumors due to PI3K and PTEN gene mutations. Powered AKT phosphorylates its key downstream effector mTOR to control various cell signaling pathways that boost cell survival, development, and proliferation, as well as energy metabolism (Dan et al. 2014). TSC2 and PRAS40, both negative regulators of mTOR activity, are known to be deactivated (phosphorylated) by AKT, which stimulates mTORC1 complex activity. Relevantly, mTORC1 activity inhibits autophagy induction (Kim et al. 2011). Being an important regulator of cell metabolism, mTOR combines signals from extracellular molecules like insulin and glucose. The mTOR disorders have been implicated in different diseases such as diabetes, cancers, and the aging process (Zoncu et al. 2011).

Plenty of studies have been conducted to elucidate the human disease mechanisms including cancers on experimental rat models. On the other hand, the aftermath of environment-dependent illnesses in wild rats is poorly understood. Therefore, the present work aims to understand the modulations of some important genes and chromosomal aberrations between the wild rats at urban and rural demographic sites of Western Anatolia. The research hypothesis is that rural habitats will be favorable in reducing the susceptibility to CRC, whereas urban circumstances (especially industrial areas) will enhance this propensity, considering the negative correlation between *Pten* and *Mtor/Akt* genes. The study is valuable for the investigation of the link between habitat differences and susceptibility to CRC, in terms of several key genes involved in one of the main intracellular signaling pathways of mammals. However, the obtained cytogenetic and molecular data are complementary and supportive of evolutionary and adaptation studies investigating the morphological, behavioral, physiological, as well as species diversity, and richness properties that reveal the negative or modifying effects of urbanization in animals at the species level.

## Materials and methods

### Habitat identification and animals

*Rattus rattus* samples were divided into two different groups according to their habitats in Western Anatolia (Bilecik province). The urban habitat group consisted of wild rats trapped in the industrial area of Bilecik city where automobile maintenance is carried out, whereas the rural habitat group comprises wild rats trapped in cow farms. The location where urban rats were trapped is in the city center, surrounded by dense housing buildings and public transportation networks. The area is also characterized by impermeable surfaces such as asphalt and concrete, very few trees and artificial green areas, chemical wastes, human foods, and garbage (Fig. 1a). The area where rural rats were trapped is represented by the Sorgun Stream (Bilecik) and natural green space (herbaceous plants such as maquis and thistle, thyme, mallow, poppy, nettle), the absence of human settlements, and cow feed stores (barley, wheat, straw, pulp) (Fig. 1b). The distance between these habitats was estimated to be approximately 11 km. Rats were caught in June 2019 using 50 live traps left in all habitats. The traps were randomly left for one night from sunset to sunrise in each habitat (≈ 1000 $m^2$). Only male rats (180–200 g) were included in the study. To reduce the captivity stress, the rats were brought to the laboratory on the day of capture, and ether inhalation anesthesia was used after a fasting period of 4 h (Gurbanov et al. 2022). Male laboratory rats (*R. rattus*) were studied as an outgroup in the comparison of gene expressions between different wild rat samples. The animals were trapped with permission (permission number: 21264211-288.04-E.3,311,026) of the General Directorate of Nature Conservation and National Parks affiliated with the Turkish Ministry of Agriculture and Forestry. The laboratory rats were kindly provided by Aydın Adnan Menderes University (project number: TPF17040).

### Chromosome analyses

The Colchicine Hypotonic Citrate method was used in the chromosome preparations from bone marrow cells (Ford and Hamerton, 1956). Giemsa-stained metaphase plates were photographed under the 100’ immersion objective. The standard karyotype idiogram of the samples and the chromosomal aberrations are visually presented.

### Preparation of tissue samples and RNA isolation

A total of 12 animal (4 animals per group) colon samples from urban, rural, and laboratory rats were used in experiments. The tissue samples (20 mg) were taken from the whole colon by sterile instrument for RNA isolation. RNA isolation was performed using the innuPREP RNA Mini Kit 2.0 (Analytik Jena GmbH) according to the manufacturer’s instructions.


**Fig. 1** Photographs of **a** urban and **b** rural stations where the rats were collected

## cDNA synthesis

cDNA was synthesized from RNAs using SCRIPT cDNA Synthesis Kit (Jena Bioscience) according to the manufacturer's instructions. Random primers (0.5 µL from 100 µM stock) were incubated for 5 min at 65 °C for RNA (10 µL from 10 ng/µL stock) binding and then the reaction media were taken on the ice. Then the prepared reaction mixture (Online resource: **Table S1**) was incubated in a thermal cycler first at 50 °C for 60 min and then at 42 °C for 10 min. After the thermal reaction, the cDNAs were stored at -20 °C.

## Real-time PCR analysis

Gene expression analysis was performed by LightCycler 480 II system (Roche Diagnostics) using the innuMIX qPCR MasterMix SyGreen Sensitive (Analytik Jena GmbH) and 96-well DNase/RNase-free plates. The reagents and gene primers (designed for this study) used in the protocol are given in the Online resource: **Tables S1 and S2**, respectively.

### Statistical analysis of gene expression data

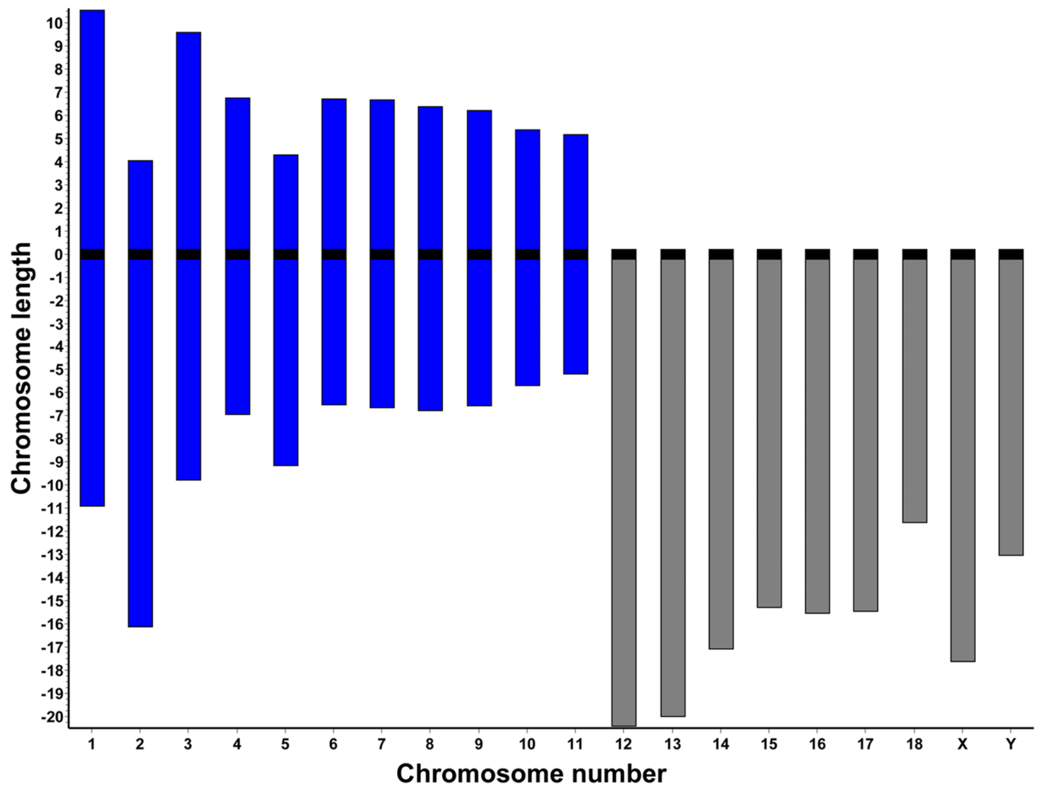
Quantity (relative) values were calculated with the help of the standard graph of Cq (ΔRn) values that changed during gene expression using the LightCycler 480 software. For each group, four individuals with three replicates, n = 12 values were used in the analysis. The differences in targeted cDNA amount were eliminated by proportioning the targeted gene quantity and reference gene (GAPDH) values in GraphPad Prism 7.0 software. The data were analyzed by applying Dunnett's multiple comparisons test with two-way ANOVA. The degrees of significance were denoted as less than or equal to $p \leq 0.0001$ ****. The Cq value of the laboratory rats' group was normalized to 1 and the detected differences in urban and rural rat groups were calculated as multiples of this value. The primer calibration curves for each studied gene are given in the Online resource: **Fig. S1**.

## Results and discussion

Cities are one of the most unnatural habitats on our planet. They provide specific physical and biological environments for organisms that are different from the conditions in their original habitat (Liker, 2020). It has been shown that cities can play an important role in modern evolution by accelerating phenotypic changes in wildlife, including animals, plants, fungi, and other organisms (Alberti et al. 2017b). Environmental changes driven by urbanization are categorized as human existence, invasive species, diet, parasites, temperature, pollution, light, noise, substrates, and roads (French et al. 2018). In this study, the chromosome and colon epithelium samples from *R. rattus* caught from two selected regions defined as urban and rural habitats were studied comparatively. The obtained cytogenetic and molecular level findings demonstrated higher chromosomal aberrations (Fig. 2), downregulation of *Pten*, and upregulation of *Mtor* and *Akt* genes (Fig. 3) in urban rats, unlike rural ones.

Data from cytogenetic analyses are shown in Fig. 2. Accordingly, 11 pairs of bi-armed chromosomes (9 pairs of metacentric/submetacentric, 2 pairs of subtelocentric) and 7 pairs of acrocentric chromosomes constitute the *R. rattus* karyotype with the normal diploid chromosome number (2n), 38. The sex chromosomes are acrocentric (Fig. 2a). Anomalies such as centric fusion, end-to-end association, dicentric chromosome, centromeric attenuation, ring chromosome, and fragment were frequently observed in samples from urban areas (Fig. 2b). When the samples from each site (urban and rural) were evaluated over a total of 100 metaphase plates, higher levels of chromosomal aberrations were detected in samples from urban areas (48%) than in rural areas (12%). Regarding urbanization and pollution, high chromosomal aberrations in industrialized areas have already been demonstrated in a wide variety of studies involving free-living rodents and humans from different countries (Monteiro Neto et al. 2010; Mitkovska et al. 2012; Yağcı and Gurbanov, 2020; Cochak et al. 2021). Pollution, which is one of the urban stress factors, is divided into chemical pollution, noise pollution, and artificial night light pollution. The most common urban chemical pollutants include various heavy metals, nitrogen oxides (NOx), nanoparticles, and polycyclic aromatic hydrocarbons (PAHs) (Isaksson, 2015). Accordingly, the chromosomal aberrations detected in urban rats indicate the toxic and mutagenic effects of possible chemical pollution on the ecosystem. The findings of the present study support the relationship between chemical pollution and chromosomal aberrations detected in previous studies.

The expression levels of *Pten, Mtor*, and *Akt* genes in the colon epithelium of rats taken from two different habitats were compared with laboratory rats (Fig. 3). *Pten* gene was overexpressed primarily in rural rats, while *Mtor* and *Akt* genes were upregulated in urban rats. Low expression rates were found for all three genes in laboratory rats. In other words, the upregulation of *Pten* suppressed the transcription of intracellular PI3K/AKT/mTOR signaling pathway components in rural rats. Whereas the loss of *Pten* transcription led to the activation of *Mtor* and *Akt* genes in urban rats. Our findings are following literature on *Pten*-associated negative regulation of PI3K/AKT/mTOR pathway known to be associated with many diseases including intestinal cancers. CRC is the most common cancer in the world and it is believed that the formation of CRC is associated with a variety of factors. Epidemiological data show that CRC is mainly affected by eating habits, physical activity, and environmental and genetic factors (Chatenoud et al. 2016). Most nutrient signals function through insulin or insulin-like growth factor (IGF) signaling pathways. On the other hand, the PI3K pathway is stimulated by integrins, growth factors and G-protein coupled receptors to forward many cellular functions (Vivanco and Sawyers, 2002). Since overstimulation of the PI3K/AKT pathway is linked to malignant activity (Phillips et al. 1998), PI3K/PTEN/AKT pathway mutations are present in a significant number of CRC cell lines (Wang and Zhang, 2014). The mTOR-mediated network of hormones, inflammation, and energy-related factors suggests that multiple ecological variables like nutritional antigens, commensal/harmful bacteria, and mucosal toxins cause a primal, recurrent, and moderate asymptomatic inflammatory response. On the other hand, extra levels of estrogen, androgens, and insulin promote more severe inflammation associated with CRC incidence (Slattery and Fitzpatrick, 2009). In 15% of metastatic CRC cases, the mutation of a




b

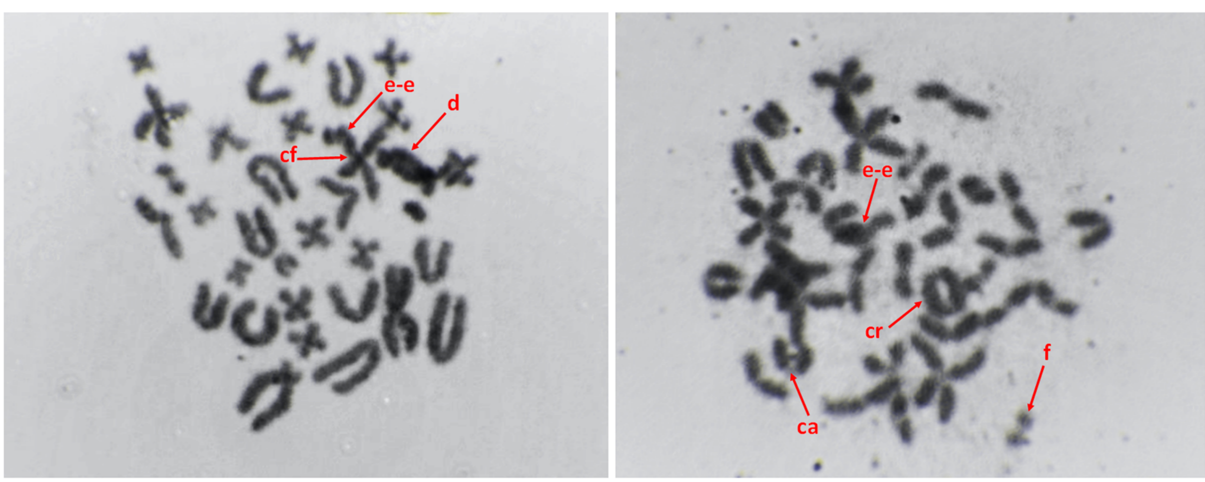



**Fig. 2** **a** Idiogram of the karyotype of *Rattus rattus* showing the morphology of the chromosomes. Blue-colored columns show the bi-armed chromosomes, whereas grey-colored columns show the acrocentric chromosomes. **b** Different types of chromosomal aberrations in the metaphase spread of *R. rattus* (urban sites). cf., centric fusion; e-e, end-to-end association; d, dicentric chromosome; ca., centromeric attenuation; cr, chromosome ring; f, fragment

PIK3CA is implicated (De Roock et al. 2011). Moreover, Cowden syndrome is consistent with PTEN mutation, which may result in an elevated lifelong risk of CRC (Pritchard et al. 2013). The relationship between mTOR signaling and other essential pathways involved in CRC pathogenesis has previously been thoroughly studied elsewhere (Wang and Zhang, 2014). In brief, the mTOR signaling is conserved in all organisms ranging from yeast to humans, and mTOR from the PI3K-related kinase family is a core protein of this pathway (Wang and Zhang, 2014). The mTOR pathway can have a direct impact on cancer development. In tissues of CRC patients, elevated mTOR mRNA and protein

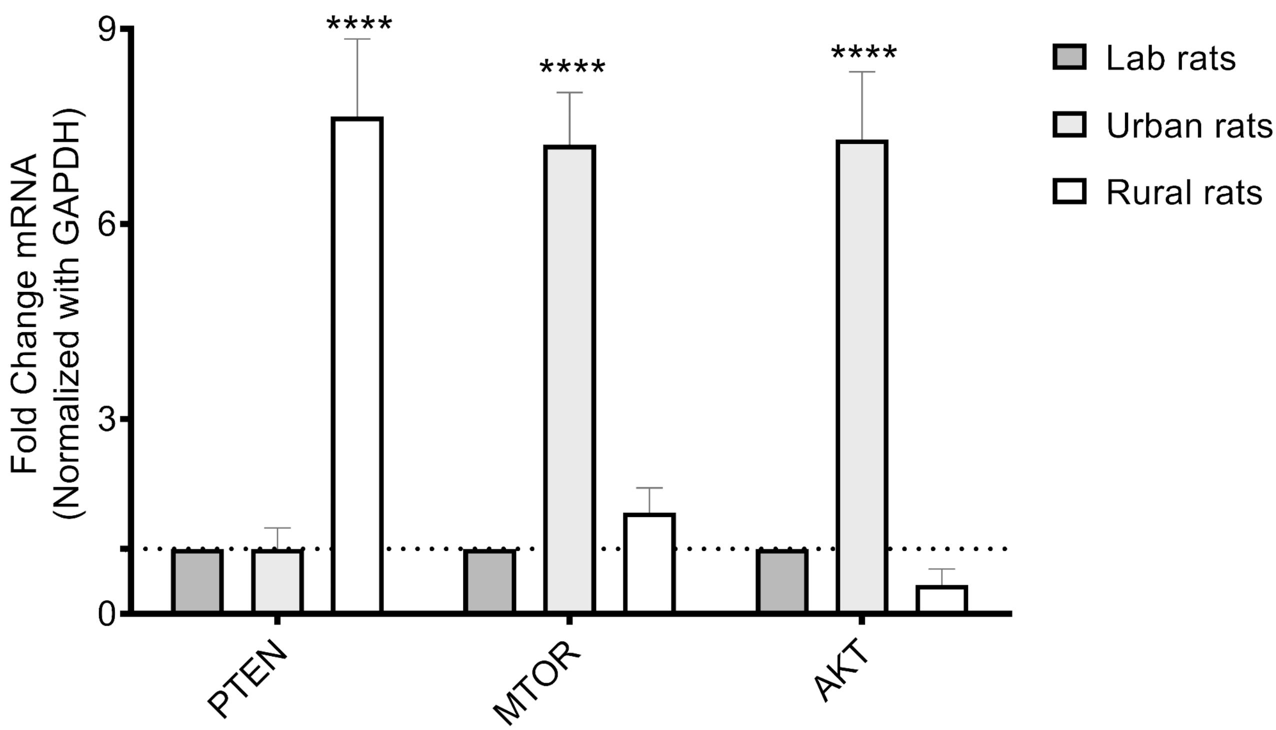



**Fig. 3** The transcription of *Pten, Mtor*, and *Akt* genes in the colon epithelium of rat groups. The degrees of significance were denoted as less than or equal to $p \leq 0.0001$ ****

levels have been found. Besides, there was a strong association between overexpression of mTOR and the inflated malignancy stage. Moreover, mTORC1/2 plays an important role in CRC metastasis through RhoA and Rac1 signaling (Gulhati et al. 2011). mTOR was suggested to lead to tumor development by inducing chromosomal instability in a genetically modified mouse model (Aoki et al. 2003). By controlling Lipin-1/SREBP1/2, metabolic processes, and ATP formation via HIF1 induction, mTORC1 enhances lipogenesis and energy metabolism, respectively. It also inhibits autophagy via ULK1 and Atg13 (Wang and Zhang, 2014). Microsatellite instability (MSI) accounts for 85% of the hereditary origin of CRC. Whereas chromosomal instability (CIN) accounts for the remaining 15%. However, several experiments have shown that the MSI and CIN pathways are not fully certain in CRC and that there is significant coupling across different routes (Imai and Yamamoto, 2008). Histopathological experiments on human colorectal tumors demonstrated that mTORC1 signaling occurs early in the carcinogenesis process and contributes to the transition of healthy cells to a malignancy morphology maintaining the basis of mTORC1-targeted therapeutics toward colorectal tumor management and rehabilitation (Zhang et al. 2009).

Worldwide incidence rates of CRC are increasing with industrialization and urbanization (Wen et al. 2018). A recent study has extensively investigated the relationship between CRC and proximity to industries and supported the hypothesis that residing near industrial areas may be a risk factor for CRC (García-Pérez et al. 2020). Research results have shown that metal, glass, chemical, food, and organic solvent industries are extremely risky. Environmental pollutants that increase the risk of CRC include benzo[a]pyrene, asbestos, nitrosamines, vinyl chloride, polypropylene, acrylonitrile, synthetic fibers, ethyl alcohol, tobacco smoke, and heavy metals such as cadmium and lead (Rogala et al. 2019). One particular common environmental pollutant is benzo[a]pyrene, which is found in cigarette smoke, many foods, car exhaust fumes, wood burning, and coal tar (Mudd et al. 2020). Besides chemical pollution, diet is the most important exogenous factor identified so far in the etiology of CRC (Migliore et al. 2011). Generally, a high intake of red and processed meats, high fructose corn syrup and unhealthy cooking methods, and calorie-dense and nutrient-deficient foods increase the incidence of early-onset CRC (Hofseth et al. 2020). In the present study, the downregulation of *Pten* and upregulation of *Mtor* and *Akt* genes in the colonic epithelium of urban rats support the previously established relationships between urbanization and cancer risk. Here, the effects of diet and environmental pollutants alone or in combination may be in question, since the food residues left in garbage bins or restaurants are low-value food sources that urban rats will encounter frequently. In addition, the region where urban rats are taken is an industrial zone where motor vehicle maintenance operations are carried out. Pollutants released into water and air by vehicle maintenance and repair businesses are classified as oil, wastewater, hazardous wastes, exhaust gas, and other pollutants. Hazardous wastes are mostly mineral oils, paints, waste organic solvents, electronic components, and waste lead-acid batteries (Guo and Wang 2018). On the other hand, fruits, arthropods, fruit with seeds, roots, flowers, grass leaves, and trace nutrients have been detected in the stomach contents of *R. rattus* residing in natural habitats (Clark, 1982). Plant foods (cattle feeds, flowers, stems, leaves, etc.) fungi, and arthropods are among the available food sources for rural rats studied herein.

The chromosomal abnormalities and changes in the expression level of some genes including *Pten* have attracted great interest in characterizing the etiology of CRC (Codrich et al. 2021). Diet and environmental factors play a leading role in the initiation and/or progression of CRC, similar to many other tumor types where they play a primary role in tumorigenesis (Grazioso et al. 2019). Changes in the expression level of *Pten, Mtor*, and *Akt* genes and chromosomal aberrations detected in urban rats indicate that these rats may be more likely to be exposed to chemical pollution and changes in the diet compared to rural populations. However, to confirm this hypothesis, a more detailed evaluation of the nutritional values and toxic chemicals emitted from industrial areas is required. This study, which reveals the negative biological responses of animals to habitats changing with urbanization, will contribute to the existing knowledge of epidemiological and mechanistic studies of human CRC from an ecological perspective.

## Conclusion

An intracellular signaling pathway, which is of particular importance in human cancers and their treatments, has been examined mutually in rats from different ecosystems for the first time in this study. The study highlights the importance of habitat diversity, particularly due to urbanization, based on the *Pten*-correlated transcriptional changes in the *Mtor* and *Akt* genes between wild rats from urban and rural ecosystems. Chromosomal aberrations seen in free-living rodents are generally associated with environmental pollution. Still, further genomic and proteomic-level research is needed to be conducted with much more wide rat groups for the extensive examination of the preliminary findings presented herein.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11756-022-01272-8.

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## Statements and declarations

**Competing interests** The authors declare they have no competing interests relevant to this article.

**Ethics approval** This study was carried out with the permission (permission number: 21264211-288.04-E.3311026) of the General Directorate of Nature Conservation and National Parks affiliated with the Turkish Ministry of Agriculture and Forestry and Adnan Menderes University Experimental Animals Local Ethics Committee-ADU HADYEK (permission number: 64583101/2017/003).
The authors declare that they have followed the ethical standards of the responsible committee.

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