Η έκφραση των ING-2 πρωτεϊνών στον διηθητικό καρκίνο του παχέος εντέρου

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The expression of ING-2 Proteins in infiltrative colon cancer

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ΠΕΡΙΛΗΨΗ

Σκοπός: ***Ο καρκίνος του παχέος εντέρου είναι ο τρίτος και ο δεύτερος, αντίστοιχα, πιο κοινός τύπος καρκίνου στους άνδρες και τους άνδρες γυναικών, σύμφωνα με νέες περιπτώσεις που αναφέρθηκαν σε παγκόσμιο επίπεδο. Σκοπός αυτής της μελέτης ήταν να συμβάλει στην κατανόηση του ρόλου του αναστολέα πρωτεΐνης γονιδίου-2 (ING2-mRNA) στον καρκίνο του παχέος εντέρου και στην αύξηση του δυναμικού του σε προγνωστικό επίπεδο.***

Μέθοδος: ***Το δείγμα της έρευνας αποτελείται από 60 ασθενείς (60% άνδρες, μέση ηλικία 73,2 ± 11,1 έτη) που υποβλήθηκαν σε χειρουργική επέμβαση για καρκίνο του παχέος εντέρου κατά την περίοδο 2008-2013 στο Γενικό Νοσοκομείο Ασκληπιείου Βούλας. Δείγματα από τον καρκινικό βλεννογονικό ιστό συγκρίθηκαν με φυσιολογικά και τα επίπεδα του ING2-mRNA ορίστηκαν ποσοτικά μέσω της Real Time Polymerase Chain Reaction (RT-PCR). Τέλος, τα επίπεδα αυτά συσχετίστηκαν με την αντίστοιχη παθολογική εικόνα και τα χαρακτηριστικά κάθε ασθενούς, χρησιμοποιώντας το πρόγραμμα SPPS V.20 για στατιστική διαδικασία. Μετά το σύνολο RNA εκχυλίστηκε, οι εκκινητές που χρησιμοποιήθηκαν καταγράφηκαν ως εξής: ING2 (sense: 5' GCAGGCAGCGGACCAGTGAA-3', antisense: 5'-CCTGCTTGGCCTTGGAGCGT-3'), GAPDH (sense: 5'-GAAATCCCATCACCATCTTCCAGG-3', antisense: 5'-GAGCCCCAGCCTTCTCCATG-3').***

Αποτελέσματα: ***Τα αποτελέσματα της μελέτης επιβεβαίωσαν τον περιθωριακό συσχετισμό που έχει ήδη τεκμηριωθεί μεταξύ της αρνητικής έκφρασης του ING2-mRNA και του σταδίου 2α της σταδιοποίησης TNM του καρκίνου του παχέος εντέρου.***

Συμπεράσματα: ***Αυτά τα αποτελέσματα οδηγούν στο συμπέρασμα ότι το επίπεδο αυτού του συγκεκριμένου mRNA μπορεί να είναι προγνωστικό του κλινικού αποτελέσματος. Απαιτούνται περαιτέρω έρευνες, τόσο για να επιβεβαιωθεί αυτή η συσχέτιση όσο και για να διασαφηνιστεί ο μηχανισμός του ING2 στον καρκίνο του παχέος εντέρου.***

Λέξεις κλειδιά: ***Καρκίνος του παχέος εντέρου, αναστολέας γονιδίου ανάπτυξης, ING2-mRNA, ποσοτική έκφραση***

ABSTRACT

Background: ***Colorectal cancer is the third and second, respectively, most common form of cancer in the male and female populations, according to new cases reported at the global level. The aim of this study was to contribute to***

the understanding of the role of the inhibitor of growth gene - 2 (ING2-mRNA) protein in colorectal cancer, raising its potential to the prognostic level.

Methods: ***The research sample consisted of 60 patients (60% men, mean age 73.2 ± 11.1 years) who underwent surgery for colorectal cancer during the period 2008-2013 in the Asklepieion Voulas General Hospital. Samples from tumor tissue mucosal were compared with normal ones and the levels of ING2-mRNA were defined quantitively through Real Time Polymerase Chain Reaction (RT-PCR). Finally, these levels were correlated with the correspond***

pathological image and characteristics of each patient, using the SPPS V.20 program for statistical process. After Total RNA was extracted, the primers used were listed as follows: ING2 (sense: 5'-GCAGGCAGCGGACCAGTGAA-3', antisense: 5'-CCTGCTTGGCCTTGGAGCGT-3'), GAPDH (sense: 5'-GAAATCCCATCACCATCTTCCAGG-3', antisense: 5'-GAGCCCCAGCCTTCTCCATG-3').

Results: ***The results of the study confirmed the marginal correlation already documented between negative expression of ING2-mRNA and stage 2a of the TNM staging of colorectal cancer.***

Conclusions: ***These results lead to the conclusion that the level of this specific mRNA can be prognostic of clinical outcome. Further research is needed, both to corroborate this correlation and to elucidate the mechanism of ING2 action in colorectal cancer.***

Key words: ***Colorectal cancer; inhibitor of growth gene; ING2-mRNA; staging, quantitative expression.***

Introduction - Aim

Colorectal cancer is the third and second, respectively, most common cancer in men and women, according to new cases diagnosed at the global level[1]. Indicatively, the estimate for 2012 was of 1.4 million new cases. The highest incidence rates have been recorded in North America, New Zealand, Australia, Europe and South Korea and the lowest in Africa and South and Central Asia. Regarding mortality from colorectal cancer, 693,000 deaths were recorded world- wide in 2012, corresponding to 8% of the total cancer deaths. Increasing tendencies in various countries have been related to changes in risk factors, such as increases in unhealthy nutrition, smoking and obesity. Conversely, there have been encouraging signs of effective management of the disease, as indicated by a reduction of mortality in countries with optimal resources[2]. Cancer initiation and development is a very complicated process, which cannot be related to a single specific factor or genetic change. At the level of genetics, cancer may be caused by the combined action of different categories of genes, through several consecutive changes and mutations. Because of the variety of changes, there are different responses to treatment schemes, leading to a remarkable heterogeneity in therapeutic effect. Oncogenic genes, tumor suppressors and microRNA (mRNA) genes of cancer cells belong to the categories involved[3,4]. The aim of this study was to contribute to the understanding of the role of the inhibitor of growth gene family (ING) proteins in colorectal cancer, as either tumor suppressor or oncogenic genes. Specifically, the study focused on the ING2-mRNA member of this family, locating its action mechanism and investigating how this mechanism defines or affects the appearance and the evolution of cancer.

Methods

For this study, firstly the role of ING proteins in several types of cancer, including colorectal, was reviewed. A systematic literature review was carried out, with a search for randomized controlled trials, cohort studies, case control studies, cross-sectional surveys, systematic reviews, and meta-analyses in the Cochrane Library, MEDLINE, and EMBASE databases. Secondly, samples of tumor tissue from 60 patients operated on in the Asklepieion Voulas General Hospital for colorectal cancer were compared with normal mucosal tissue; the levels of ING2-mRNA were quantified using real time polymerase chain reaction (RT-PCR). Thirdly, the levels of ING2-mRNA were correlated with the corresponding histopathological image and characteristics of each patient, using the SPPS V.20 program for statistical analysis.

Total RNA was extracted using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. RNA concentration and quantity were assessed by absorbency at 260nm; 2μg of total RNA was reverse transcribed into first-stand cDNA with Moloney murine leukemia virus reverse transcriptase (Invitrogen, NY, USA) and oligo (dT)12-18 primers (Invitrogen, NY, USA) in a 20μl reaction. RT-PCR was performed with GAPDH as an inter- nal control. The primers used were listed as follows:

ING2 (sense: 5' GCAGGCAGCGGACCAGTGAA-3', antisense: 5'-CCTGCTTGGCCTTGGAGCGT-3'), GAPDH (sense: 5'-GAAATCCCATCACCATCTTCCAGG-3', antisense: 5'-GAGCCCCAGCCTTCTCCATG-3'), and the corresponding PCR products were 132bp and 120bp, respectively. PCR amplification was programmed for 1 cycle at 95 oC for 4min, 30 cycles of 95 oC for 30s, 58 oC for 45s and 72 oC for 30s, followed by a final extension of 7min at 72 oC. The amplification products were electrophoresed through 3% (w/v) agarose gels and visualized with ethidium bromide (Sigma, St. Louis, MO, USA) on top of a UV light illuminator. The density estimation of ING2-mRNA concentration was performed using Quant one analysis image software and normalized with GAPDH

Results

The research sample consisted of 60 patients (60% men and 40% women) who underwent surgery for colorectal cancer during period 2008-2013 (75% of these were pro grammed operations). Their average age was 73.2 years (min 32, max 92 years, SD 11.1 years, with 41.7% aged between 71 and 80 years.

The main areas of excision were the rectum (41.7%) and descending colon (16.7%), and the ascending colon also presented also a high frequency (15%). Regarding the type of excision, low anterior excision and right colectomy were the most commonly applied (33.3% and 21.7%, respectively).

No correlation (x2 criterion) was demonstrated between the area of excision and the proportion of positive lymph nodes (p=0.188). Conversely, a marginally statistically significant correlation was found between the type of excision and the proportion of positive lymph nodes (p=0.049).

The area and type of excision with the highest frequencies were related to the 0-0.01 range of positive lymph nodes. According to the Kolmogorov-Smirnov criterion, the quantitative expression of ING2-mRNA can be considered approximately normal (p=0.016). ING2-mRNA expression and staging by the Dukes system were not significantly correlated (p=0.32). Negative expression of ING2-mRNA showed a relationship of higher significance with stage B (Dukes) than with other categories (expression «-1 to -0,6» and «-0,59 to -0,2» for 11 and 14 cases respectively). Similar results were observed for correlation between Astler- Coller staging and quantitative expression of ING2-mRNA (p=0.337) and for the relationship between stage B2 and negative expression of ING2-mRNA (expression «-1 to -0,6» and «-0,59 to -0,2» for 10 and 13 cases respectively). ING2- mRNA expression and staging by the TNM system were significantly correlated (p=0.003), with higher allocation in cases of stage 2a (expression «-1 to -0,6» and «-0,59 to -0,2» for 10 and 13 cases respectively). In order to explore the differences in ING2-mRNA expression, corresponding box plots were created. Symmetry was observed for stages B, B2 and 2a for systems Dukes,Astler- Coller and TNM respectively. In addition, these stages showed the strongest relationship between negative expression of ING2-mRNA and allocated cancer cases. This symmetry confirms the previously documented negative expression of ING2-mRNA in the corresponding cancer stages.

Discussion

The literature review revealed variation in study results concerning positive or the negative overexpression of ING2-mRNA. Both forms of expression were indicated in several different studies on the appearance and evolution of many cancer types. Reduced expression of the ING family has generally been documented in cancers including lung, breast[5], stomach[6, 7], esophagus[8], blood, brain[9] and head and neck squamous cell carcinoma (HNSCC)[10]. In the case of ING1, especially, deficiency in its ex- pression has been correlated with both earlier onset and higher frequency of lymphoma (knockout trials in mice)[11], loss of nuclear p33ING1b in cases of melanoma, seminoma, thyroid carcinoma, porous breast carcinoma and acute lymphoblastic leukemia (ALL)[12]. These findings on ING1 are of importance to the present study because of the high proportion of homology between ING1 and ING2 (58.9%)[13].

Reduced expression of ING2 has been reported in skin cancer[13] and HNSCC[14]. Specifically, reduced nuclear expression of ING2 has been documented in human melanoma[13], non-small cell lung cancer (NSCLC), with a higher frequency in adenocarcinoma[15]. Reduced levels of ING2 have also been reported in cases of hepatocellular carcinoma[16]. For HNSCC in particular, ING2 is referred to as downregulated[17].

In a series of 79 cases of primary melanoma[13], the nuclear expression of ING2 was found to be significant reduced in the radial growth phase (RGP) and the vertical growth phase (VGP). No correlation was demonstrated between the expression of ING2 and specific patient characteristics, such as sex, age, stage, survival rate or tumor characteristics, including thickness, subtype and position. One relevant study 18 found that the expression of ING2-mRNA was reduced in 6/7 cellular series of lung cancer. Upregulated expression of ING2 has been identified in colorectal cancer[19], Burkitt lymphoma and cervical cancer[20]. Specifically, for colorectal cancer[19], in a total sample of 39 patients (average age 59.8 years, with TNM staging) the ING2-mRNA level more than double that in normal mucosa in 45% of the cases studied.

In similar studies[17], reductions of 50%-76% in mRNA in the ING3-5 genes for HNSCC were recorded. In addition,

a high expression of nuclear ING4 in HNSCC was associated negatively with TNM staging and positively with lymph node metastasis. In the case of ING5, its nuclear expression, and also abnormal cytoplasmic expression, was correlated with differentiation of HNSCC[10].

The high degree of heterogeneity between these studies should be emphasized, related to many parameters of the study process, including the type of the sample (patients or cellular lines), the mechanism of ING action and interaction with cancer, the mutation of ING genes and the changes in ING expression. According to the mechanism of ING action, the contribution of ING in the appearance and development of cancer has been variously attributed to its binding to RPBI -mSin3A-HDAC complex, to MMP13 promoter, and to upregulation of MMP-13 expression[19], and also to ING2 function as a novel mediator of transforming growth factor (TGF)-β-dependent responses in epithelial cells[21].

In general, the ING family has been identified as tumor suppressor genes, involved in the downregulation of the cell cycle and the reforming of chromatin[17]. Conversely, ING1 and ING2 are thought to play an oncogenic role in some types of cancer, similarly to that of TGF-/3, and specifically their movement from nucleus to cytoplasm is considered to be a critical point in the process of carcinogenesis[17]. A relevant study on melanoma[18], detected no correlation between nuclear expression of ING2 and the phase of tumor development, leading to the conclusion that reduced ING2 expression is involved more in initiating the process of appearance of melanoma, rather than its subsequent development. Finally, most of the studies ended up concluding that the specific mechanism of regulation needs further research.

The following table summarizes and compares the results obtained in this study with the other analogous clinical studies.

Table: Summary of studies on the role of the inhibitor of growth gene family (ING) proteins in the initiation and development of cancer.

iNG	iNG expression	Type of cancer	reference
iNG2	Downregulated Downregulated	lymphoma Melanoma, thyroid carcinoma, porous breast carcinoma and acute lymphoblastic leukemia	[11] [12]
iNG2	Downregulated	Skin	[13]
		HNScc	[14]
		NSclc	[15]
		Hepatocellular	[16]
		lung	[18]
		HNScc	[17]
	Upregulated	colon and rectum	[19]
		lymphoma Burkitt, cervical	[20]
iNG3	Downregulated	HNScc	[17]
iNG4	Downregulated	HNScc	[17]
iNG5	Downregulated	HNScc	[17]
HNSCC= head and neck squamous cell carcinoma			

Conclusions

Review of the relevant literature on the quantitative expression of ING2-mRNA in cancer, and especially in colorectal cancer, reveals a high degree of heterogeneity. A general convergence emerges towards the relationship of negative expression of ING2-mRNA with cancer. This relationship was also concluded from the results in the present study, and in particular for stage 2a of TNM staging for colorectal cancer. According to these results, the selected mRNA level can be used prognostically for clinical outcome (databases with matrix correlations for several types of cancer and their stages, etc.). Further research is needed, not only for confirmation of this correlation but also for increasing the understanding of the complete mechanism of ING2-mRNA action in cancer.

Acknowledgements

We would like to thank Dr. Maria Gazouli, PhD, Associate Professor of Molecular Biology of the School of Medicine of the National and Kapodistrian University of Athens for her contribution with the experiment molecular processing, as well as all the patients who participated in the study.

Ethical Approval

Approval was ensured from the medical board of the hospital committee for the research project. Written informed consent was obtained from all the patients for publication of their personal medical data.

Conflict of Interest

The authors declare that they have no conflict of interest. They also have full control of all primary data and they agree to allow the journal to review their data if requested.

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