RESEARCH COMMUNICATION

Elevated Serum Thymidine Kinase 1 Predicts Risk of Pre/Early Cancerous Progression

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Abstract

Background: Serological thymidine kinase 1 (STK1) is a reliable proliferation marker for prognosis, monitoring tumour therapy, relapse and detection of malignancies. In this study we investigate the use of STK1 in health screening. Methods: The concentration of STK1 was determined by a sensitive dot blot ECL assay in 8,135 persons participating in a health screening program. Results: The frequency of persons with elevated STK1 (>2.0 pM) was 1.1%, representing diseases linked to pre/early cancerous progression. One person with malignancy (gastric carcinoma) was found among persons with elevated STK1, but none of persons with normal STK1 values. There was a significantly higher frequency of persons with moderate/severe type of hyperplasia of breast and prostate expressing elevated STK1, compared to persons with normal STK1 values. No significant difference was found concerning mild hyperplasia. Of persons with elevated STK1, 89.2% had diseases linked to risk for pre/early cancerous progression, compared to 41.2% of persons with normal STK1 values. Among the persons with elevated STK1 values, one developed liver carcinoma after 13 months and five persons showed progression in their disease within 19 months (breast and prostate hyperplasia, HBV infection). Conclusion: Serological TK1 may be a reliable marker for risk assessment of pre/early cancerous progression.

Keywords: Serological thymidine kinase 1 (STK1) - health screening - premalignancy - malignancy - hyperplasia of breast - prostate benign hyperplasia (PBH)

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Introduction

Cancer is a leading cause of death in China and the number of affected individuals increases for each year, although different methods of treatment for cancer, e.g. chemotherapy, endocrine therapy, radiotherapy and surgery, have been improved tremendously during last decades. These circumstances, and that about 98% of health screening tested persons show diseases or illness in China (Chen et al., 2008; Zhang et al., 2010), indicates that health screening is of great significance to improving the quality of life for a majority of people.

Cancer, a chronic disease of abnormal proliferation cells, could be treated well if discovered in its early development stage. Mutations in certain genes associated with cell growth regulation leads to un-controlled proliferation and thus development of malignancies, which may be take 10-30 years. Non-invasive serological methods for early detection of tumors may be give information of potential malignancies before the assessment by imaging techniques and thus increases the possibility to cure patients. The tumor markers are compounds produced by tumor cells or by other cells within the body in response to the malignancy or certain benign tumor conditions. It may be detected within bodily fluids, such as peripheral blood or plasma, urine, saliva, sputum, cerebrospinal fluid, or effusions. Although numerous tumor markers have been identified with its own clinical characteristics (Cigna, 2008), a review of the literature states that measurements of most tumor markers levels alone are often insufficient to diagnose malignancies in health screening of the following reasons: 1) tumor marker levels can be elevated in person with benign condition; 2) tumor markers levels are not elevated in every person with malignancy, especially in the early stages of the malignant disease; 3) many tumor markers are not specific to a particular type of tumor; and 4) the level of a specific tumor marker can be elevated by more than one type of tumor (Cigna, 2008; Pissaia et al., 2009). As a result of these findings, guidelines for the use of tumor markers in a range of malignancies have been developed. These guidelines have been published by the American Society of Clinical Oncology (ASCO), the American Cancer Society (ACS), the National Cancer Institute (NCI), and the National Comprehensive Cancer Network (NCCN).

Thymidine kinase 1 (TK1) activity in serum is a tumor

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growth-related marker used for lymphoma and leukemia (Kallander et al., 1984; Hallek et al., 1992; O'Neil et al., 2001; Topolcan et al., 2008) since 1980, but also to some extent in breast carcinoma patients (Broet et al., 2001; O'Neill et al., 2001; Topolcan et al., 2008). No immunohistolology of TK1 was available due to lack of anti-TK1 antibodies. The development of new generation of chicken polyclonal (IgY) and mouse monoclonal anti-TK1 antibodies by us (Wu et al., 2003) improved extensively the clinical use of TK1 to almost all type of solid human tumors, both for serum (Zou et al., 2002; Wu et al., 2003; He et al., 2005; Li et al., 2005; He et al., 2006; Zhang et al., 2006; Xu et al., 2008; Zhang et al., 2008; Carlsson et al., 2009; Luo et al., 2009; Xu et al., 2009; Chen et al., 2010; He et al., 2010; Li et al., 2010; Pan et al., 2010) and immunohistology (Wu et al., 2003; Luo et al., 2010) analysis. With new generation of anti-TK1 antibodies, TK1 concentration in serum (STK1) is now useful for prognosis (He et al., 2006; Luo et al., 2009; Li et al., 2010; Pan et al., 2010), treatment monitoring (Zou et al., 2002; Li et al., 2005; Zhang et al., 2006; Xu et al., 2008; Carlsson et al., 2009; Luo et al., 2009; Xu et al., 2009) and discovery of recurrence (He et al., 2006; Zhang et al., 2006; Xu et al., 2008; Carlsson et al., 2009; Chen et al., 2010) of a number of solid human tumors in addition to lymphoma and leukemia, showing an increasingly important role of TK1 in clinical setting. STK1 seems also to be useful for early screening of hyperplasia/neoplasia (He et al., 2005; Chen et al., 2008; Xu et al., 2008; Zhang 2008; Zhang et al., 2010) and thus could be used as a warning system for development of malignancies later in the life. Since 2005, 20,853 persons participating health screenings have been investigated using the commercial available STK1 kit (SSTK Ltd., Shenzhen, China) (Chen et al., 2008; Zhang et al., 2010). In a health screening of 11,278 persons living in Changsha, China, with occupation in offices, 0.5% were found to have a STK1 level >2.0 pM, a STK1 level regarded as an increasing risk to develop malignancies years later (Chen et al., 2008). In another health screening study of 8,869 persons working at an oil company in Jilin, China, the number of persons with STK1 >2.0 pM was 5.8%. The higher number of persons was likely due to the working conditions of pollutions that increase the risk to develop diseases, including cancer. The number of persons in the STK1 elevated group that directly handled the oil products (drilling, prospection, transportation) was also significantly higher compared to persons working in the office (Zhang et al., 2010).

In this study we analyze data obtained of a health screening of ordinary city residents living in Fuzhou, south-east of China of the Fujian province. The TK1 antibodies used are commercially available as safety laboratory kits, (SSTK Ltd., Shenzhen, China) showing high sensitivity and specificity with no cross-reactivity of human serum. The Receiver Operation Characteristic (ROC) value, a statistical method commonly used when assessment of health screening methods, was found to be 0.941 (Xu et al., 2008). Thus, the STK1 is a reliable marker providing a high degree of discrimination between pre-malignant/malignant patients and healthy individuals. This study was performed in order to confirmed earlier health screening studies (Chen et al., 2008; Zhang et al., 2010), indicating that STK1 give information of the risk to develop malignancies later in life. The new subject in this study is an investigation of the relationship between STK1 and different degree of hyperplasia of breast and prostate tissues, showing significantly higher frequency of persons with moderate/severe hyperplasia in the group of elevated STK1 values.

Materials and Methods

Health screening

Health screening of 8,135 persons (man 4,085, woman 4,050) were performed at Fujian Second Hospital Health Centre, Fuzhou, China, 2008-2009. The persons performing the health screening test were covered by medical insurance and represented mainly people living and working in Fuzhou city. The type of occupations was as follow: company 38.7%, office 24.4%, teachers 11.3%, clinical doctors 11.1%, retired 7.3%, lawyer 5.7%, and others 1.5%. The mean age was 47.8±9.8 with a range of 11-89. The persons were tested for STK1 in addition to three different medical tests systems routinely used at the Health Centre: 1) blood and urine tests; 2) imaging examination; and 3) physical examination.

Diagnosis

Persons participated the health screening were diagnosed for malignancies, premalignancies such as polyploid lesions; moderate/severe hyperplasia (mainly breast and prostate hyperplasia and benign hyperplasia detected by enlargement of the gland discovered by ultrasound and urination); moderate/severe of cervical erosion, gastric ulcer superficial atrophic gastritis and fatty liver; another diseases including benign lesions, over weight, anemia (such as marrow insuiltration, chronic disorders), hepatitis B virus-positive (HBV), chronic inflammations (such as Helicobacter pylori-positive, papilloma virus, etc.) with abnormalities of blood and urine biochemical changes (Underwood, 2004), that might link to the risk of development of permalignant/ malignant diseases (Underwood, 2004). Acoording to request of the Health Centre, the persons were also tested for heart diseases (cardiae enlargement, coronary disease, arteriosclerosis, myocardial infarction, irregular heart beat), blood pressure, stone (kidney, gall bladder) and tuberculosis.

Follow up

Persons with elevated STK1 values were followed up (57/78) between five and 33 months after the first STK1 test regarding any changes of the diagnosis: 0-6 months, 13 persons; 7-12 months 5 persons; 13-18 months 24 persons; 19-24 months 9 persons; 25-33 months 6 persons. Of these persons, STK1 were also determined in some of them during the follow up time period (32/57).

TK1 in serum

The concentrations of STK were measured by using a commercial Kit based on an enhanced chemiluminescent (ECL) dot blot assay as described by the manufacturer

(SSTK Ltd., Shenzhen, China). Samples comprising 3μl of serum were directly applied to nitrocellulose membrane in duplicates. The serum samples were probed with anti-TK1 chicken IgY antibody raised against a peptide (residue 195-225, GQPAG PDNKE NCPVP GKPGE AVAAR KLFAPQ). TK1 peptide was dotted at different concentrations (20, 6.6 and 2.2 pM) as an extrapolation standard. The intensities of the spots on the membrane were determined by a CIS-l Imaging System (SSTK Ltd., Shenzhen, China). From the intensities of the TK1 standard of known concentrations, the STK1 was calculated and expressed as pM. For detail description of the STK1 assay, see reference (Chen et al., 2008). In this study, 2.0 pM of STK1 was used as a threshold value, based on a receiver operating characteristic (ROC) analysis of healthy persons and patients with different types of malignancies, showing a ROC-value of 0.94 (Xu et al., 2008). Persons expressing STK1 values of less than 2.0 pM were denoted as the "Normal STK1 Group", regarded as a low risk of developing malignancies, while persons expressing STK1 values ≥ 2.0 pM were denoted as the "Elevated STK1 Group", likely representing individuals with increasing risk of premalignancy/malignancy progression.

Statistical analysis

The mean values of STK1 levels were calculated by a mean ± standard deviation program (Microsoft Excel). For comparison of STK1 concentration levels among the different groups of persons investigated, Kruskal-Wallis and Chi-square tests were used (Analysis-It, ver 2.2, UK). P-values ≤ 0.05 were regarded as statistically significant.

Results

STK1 distribution

The distributions of the STK1 values of the Normal and the Elevated STK1 Groups are shown in figure 1. The mean STK1 value of the Normal STK1 group (n=8,048) was 0.8 ±0.4 pM, while the mean STK1 value of the Elevated STK1 Group (n=87) was 3.3±0.9 pM. Of the Normal STK1 Group, 68.9% persons had STK1-values ≤ 1.0 pM. The percentage of persons in the Elevated STK1 Group of the total number of persons participating the health screening was 1.1% (87/8,135).

Diseases of Normal and Elevated STK1 groups

The persons participating in the health screening were subdivided into ten major groups depending of diseases or health conditions (Table 1). More than 95% of the persons had more than one type of disease (Table 2), and thus, the contribution of STK1 is from more than one type of disease. In each subgroup of disease the total number of persons is given. The number of cases is higher than the number of persons, since one person had more than one disease (Table 2).

In the Normal STK1 Group, the highest frequency of person was found in the subgroup of low risk diseases(HBV score 2, 4, 5, and 2, 5, mild type of hyperplasia, cervical erosion/gastric ulcer and fatty liver (47.0%), followed by

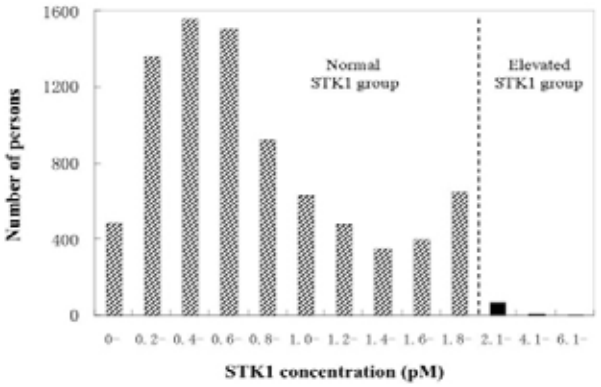

Figure 1. Distribution of STK1 of Normal and Elevated STK1 Groups

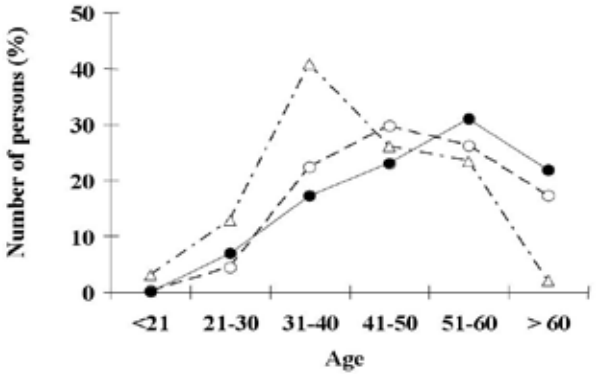

Figure 2. Age Distributions of Illness-free Persons (△), Persons of Normal STK1 Group (○) and Persons of Elevated STK1 Group (●)

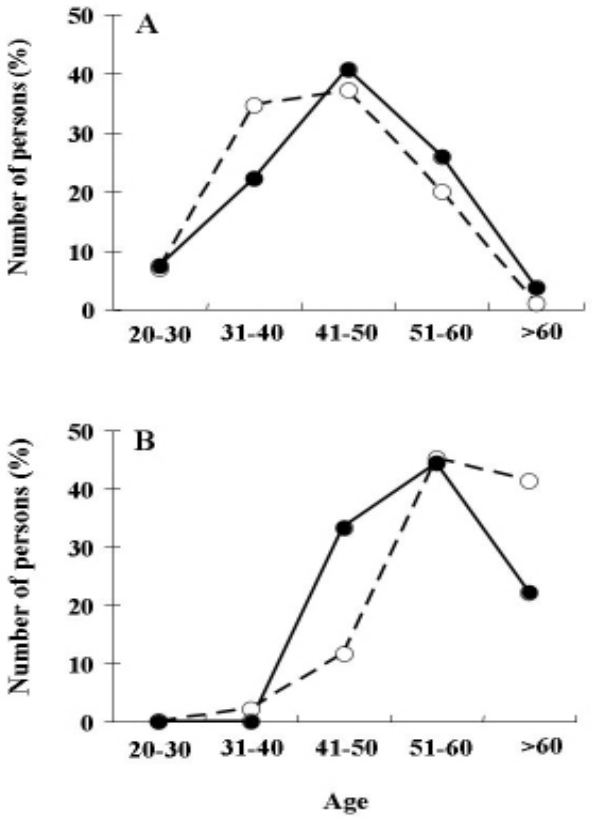

Figure 3. Age Distributions of Woman with Breast Lobular Hyperplasia (A) and of Man with Prostate Benign Hyperplasia (B) of Normal STK1 (○) and Elevated STK1 (●) Groups. The Hyperplasia of Woman and Man was of Moderate/Severe Type

Table 1. Number of Persons in the Various Diseases Subgroups of the Normal and Elevated STK1 Groups. Subgroups

Type	Normal STK1 Group	Elevated STK1 Group	X^2	DF	P-value
*Malignant	-	1/78 (1.3%)	-	-	-
*Pre-Malignant	301/8,048 (3.8%)	10/78 (12.8%)	14.8	1	0.001
Breast Hyperplasia, Mild Type	287/4,004 (7.2%)	1/46 (2.2%)	1.56	1	0.21
*Breast Hyperplasia, Moderate/Severe Type	1535/4004 (38.3%)	28/46 (60.9%)	3.73	1	0.05
Prostate Hyperplasia, Mild Type	307/4044 (7.6%)	2/32 (6.3%)	0.07	1	0.79
*Prostate Hyperplasia, Moderate/Severe Type	428/4044 (10.6%)	11/32 (34.4%)	12.4	1	<0.001
Benign	775/8.048 (9.6%)	11/78 (14.1%)	1.4	1	0.24
HBV High Risk	158/8.048 (2.0%)	3/78 (3.8%)	1.33	1	0.25
Fatty Liver	254/8,048 (3.2%)	4/78 (5.1%)	0.9	1	0.34
*Chronic Diseases	724/8,048 (9.0%)	2/78 (2.6%)	3.49	1	0.06
*HBV Low Risk, Low Prol. Tissue etc.	3,779/8,048 (47.0%)	8/78 (10.3%)	20.3	1	<0.001
*Illness-Free	203/8,048 (2.5%)	-	-	-	-

1) malignancy; 2) premalignancy (moderate/severe degree); 3) mild/moderate/severe hyperplasia of lobular breast; 4) mild/moderate/severe prostate benign hyperplasia; 5) benign lesions; 6) HBV-infection high risk (score type 1,3,5 or 1,4,5); 7) moderate/severe fatty liver; 8) chronic diseases (obesity, chronic gastritis, anemia, liver function disorder, blood abnormalities of white blood cells and erythrocytes); 9) low risk diseases (HBV score types 2, 4, 5 or 2, 5, mild degree of hyperplasia, cervical erosion, gastric ulcer, and fatty liver); and 10) illness-free (no detectable diseases or illness). The statistical calculations were done by Chi-square test. The stars (*) indicate groups of statistical significant differences.

hyperplasia (31.8%, breast +prostate hyperplasia), benign (9.6%), chronic diseases (9.0%), moderate/severe type of fatty liver (3.2%), and HBV score1,3,5 or 1,4,5 (2.0%). Few persons with premalignancies were found (3.7%), and no persons with malignancies. Only 2.5% of the people participated in the health screening were regarded as disease and illness-free. The mean STK1 values of these major groups of persons were less than 1.0 pM (0.7-0.9 pM) (Table 3).

In the Elevated STK1 Group of 87 persons, only 78 persons were tested for both STK1 and the other physiological parameters performed in the health screening, and thus, the results from 78 persons are given. As compared to the Normal STK1 Group, significantly higher number of persons were found with premalignancies (moderate/severe degree, 12.8% versus 3.7%, p<0.001), moderate/severe type of hyperplasia of lobular breast (60,9% versus 38,3%, p=0.054) and prostate benign hyperplasia (34.4%% versus 10.6%, p<0.001), but significantly less number of persons with low risk diseases (HBV score 2, 4, 5 or 2, 5, mild hyperplasia, cervical erosion, gastric ulcer, and fatty liver, 10.3% versus 47.1%, p>0.001). There was also less number of persons with moderate/severe chronic disease as compared to persons with Normal STK1 values (2.6% versus 9.0%), however, not significantly different (p=0.062). Thus, 89.2% of the persons of the STK1 Elevated Group had diseases related to processes of malignancie's risk. (Table 1). There was no significantly difference in the number of persons of mild hyperplasia of breast and prostate of the Normal and the Elevated STK1 groups (Table 1). One person with gastric carcinoma was found in the Elevated STK1 group. The mean STK1 values of the various subgroups of persons in the Elevated STK1 Group were higher than the corresponding STK1 values of the Normal STK1 Group, in the range of 2.8 to 4.3 pM (Table 3).

Follow up

In the Elevated STK1 group one person died due to gastric carcinoma 16 months after the time of the first STK1 test (Table 4). One person was diagnosed liver carcinoma 13 months after the first STK1 test, with an increase in the STK1 value from 2.9 pM to 28.6 pM (Table 4). Five persons showed progression in their diseases (breast and prostate hyperplasia, HBV infection) during the folWlow up time period (Table 4). These five persons received treatments (surgery, chemotherapy, HBV treatment). In 27 persons, STK1 returned to normal values within 33 months after received treatments and/or changes in their life style. Of these persons, 23 did not show any changes in their diagnosis during the follow up time period (Table 4).

Age distribution

The mean age of persons of the Elevated STK1 Group was significantly higher compared to persons of the Normal STK1 Group, and also between persons of the Normal and the Illness-free Groups, as well as

Table 2. Number of Cases with Different Types of Diagnosis in the Various Diseases Subgroups of Persons in the Elevated STK1 Group

Type	Malig.	Premalig.	Hyperpl.	Benign	HBV	Fatty Liver	Chronic Diseases.
Malignant, n=1	1	0	1	0	0	0	1
Premalignant, n=11	0	11	4	6	1	1	8
Hyperplasia, n=42	1	4	39	13	10	0	19
Benign, n=28	0	5	13	28	7	0	18
HBV, n=16	0	4	11	10	16	0	1
Fatty Liver, n=2	0	0	0	0	0	2	1
Chronic Inflam, n=24	1	8	16	18	1	0	24

Table 3. STK1 Values (pM) in the Various Diseases Subgroups of the Normal and Elevated STK1 Groups of Persons. Mean Values ± Standard Deviations, n=number of Persons

Type	Normal STK1 Group	Elevated STK1 Group
Malignant	-	3.3, n=1
Pre-Malignant	0.9±0.5, n=301	4.0±2.3, n=12
Hyperplasia	0.9±0.5, n=1,850	3.0±0.5, n=36
Benign	0.8±0.4, n=775	3.2±0.8, n=11
HBV High Risk	0.9±0.4, n=158	2.9±0.8, n=4
Fatty Liver	0.7±0.4, n=254	4.3, n=1
Chronic Diseases	0.8±0.4, n=724	3.3±1.8, n=5
Low risk Diseases (HBV Low Risk, Low Prolif. Tissue etc.)	0.8±0.5, n=3,779	2.8±0.5, n=8
Illness-Free	0.9±0.6, n=203	-

Table 4. Follow Up of Persons of the STK1 Elevated Group. The Follow Up Time was Five to 33 Months

Type	Number of Patients	Notes
Death	1	Diagnosed Gastric Carcinoma (STK1 3.3 pM), Died after 16 Months.
New Malignancy	1	First Test of STK1 2.9 pM, after 13 Months Diagnosed Liver Carcininoma (STK1 28.6 pM).
Disease in Progress	5	Breast hyperplasia (n=2, 6 and 13 Months) Prostate Hyperplasia (n=2, 6 and 19 Months) HBV (n=1, 13 Months)
Patients in Treatment	5	Surgery, Chemotherapy, HBV Treatment, Change of Life Style.
Disease Unchanged	23	Continue to Follow Up

between persons of the Elevated STK1 and the Illness-free Groups (Table 5, Figure 2). The mean age of all man of the Elevated STK1 Group was also significantly higher compared to the man of the Normal STK1 Group (Table 5). No such differences in the mean age were seen in the all woman group (Table 5). In woman with lobular hyperplasia of moderate/severe type, a significantly higher mean age was found in the Elevated Group, compared to the Normal Group (Table 5, Figure 3A). No significantly differences were found among the man with prostate benign hyperplasia of moderate/severe type between the Elevated STK1 and the Normal groups (Table 5, Figure 3B).

There were no significantly differences in STK1 values between young, middle or old persons of the illness-free group (Table 6), showing that the significantly differences in the STK1 values between the Normal and Elevated STK1 groups were not due to increasing ages.

Discussion

TK1 is an enzyme that allows the cell to utilize an alternate metabolic pathway for incorporating thymidine into DNA, and thus, TK1 is expressing in proliferating cells. Several clinical investigations show that elevated STK1 values indicate active tumor growth (Wu et al., 2003; Topolcan et al., 2008; Munch-Pettersen 2010). On the other hand, healthy individuals display low level of STK1, but some healthy persons could also show transient increases of STK1 when receiving acute illness (infection, inflammation) or perform other physiological changes (menstruation, blood donor). The mechanisms behind the differences in the STK1 level between healthy persons and persons with malignancies are not fully understood. However, it is likely that higher concentration of STK1of tumor patients is due to release of TK1 to the blood when tumor cells in S-phase/G2 stage of the cell cycle are disintegrated. The intracellular concentration of TK1 is high in later stages of the cell cycle, but low in G1 stage (Topolcan et al., 2008; Munch-Pettersen, 2010). Normal proliferating cells die in G1 stage of the cell cycle. Results also indicate that a factor in serum affects the stability of TK1 in serum differently in healthy persons and in tumor patients (He et al., 2005). However, it can not be excluded that TK1 is excreted in a different way from normal and tumor growing cells.

In this study we found that the frequency of persons with STK1 level ≥2.0 pM was 1.1%. Since most of the

Table 5. Age Distributions. The Hyperplasia of Woman and Man is of Moderate/Severe Type. The Statistical Calculations were done by Kruskal-Wallis Test. Mean Values ± Standard Deviations, n=number of Persons

Type	Normal STK1 Group	Elevated STK1 Group	Illness-Free	x^2	DF	P	Note
All Cases	47.7±11.8 n=8,048	50.5±10.2 n=78	40.5±10.3 n=202	5.05	1	0.03	Normal vers Elevated
				70.6	1	<0,001	Normal vers Illnes-Free
				38.7	1	<0,001	Illness-Free vers Elevated
All Woman	47.9±11.9 n=3,994	49.2±11.5 n=52	-	0.89	1	0.34	Normal vers Elevated
Woman Lubular Hyperplasia	42.9±8.5 n=1,408	45.1±9.0 n=27	-	3.94	1	0.05	Normal vers Elevated
				2.25	1	0.13	Elevated Woman vers Elevated Hyperplasia
All Man	47.9±11.9 n=4,046	52.5±13.7 n=35	-	6.03	1	0.01	Normal vers Elevated
Man Benign Hyperplasia	59.2±9.0 n=413	56.7±12.1 n=9	-	0.71	1	0.40	Normal vers Elevated
				0.25	1	0.62	Elevated Man vers Elevated Hyperplasia

Table 6. STK1 Values in Relation to Age of Persons of the Illness-free Group. Mean Values ± Standard Deviations, n=number of Persons

Ages (years)	STK1 (pM)	
0-20	1.0 ±0.7	n=6
21-30	1.0±0.5	n=24
31-40	0.9±0.5	n=83
41-50	1.1±0.6	n=53
51-60	0.9±0.6	n=31
>60	0.9±0.6	n=4

malignant patients (65-90%) express a STK1 concentration >2.0 pM (He et al., 2005), it is likely to predict that persons with elevated STK1 values, but no visible tumor, have tumor not yet discovered or have an increased risk to develop malignancies later in their life. The frequency of persons with STK1 ≥2.0 pM in this study (1.1%) is higher than the cancer incident rate of about 0.2-0.5% in China (Yang et al., 2005), indicating that not all of these persons showing elevated STK1 values will develop malignancies in the future, but give a risk warning that some of them will do it. This is may be indicated by the follow up study. Among the persons showing Elevated STK1 values, only 2 persons showed malignancies and another five persons reported progression in their malignancy linked diseases (hyperplasia and HBV infection). This corresponds to about 0.1% of the persons participating the health screening, which is closer to the cancer incident rate of 0.2-0.5%. However, it is important to note that this follow up is just preliminary, and final conclusion is able to draw only after long time follow up, i.e. 10 - 15 years. It is also important to note that this type of health screening may be not included those persons with advance malignancy diseases, since those persons contact hospital directly for examination. Finally, the present study is not a random study, but a selective one, included only a group of persons living and working in city. It is know that the cancer incident rate differ depending on occupation and living condition.

The frequency of persons with STK1 ≥2.0 pM reported in this study is similar found in a health screening study of 11,278 persons working in office of 0.5% (Changsha, China) (Chen et al., 2008). A majority of persons in that study (83%) expressing STK1 ≥2.0 pM showed diseases related to risk development of malignancies and a significantly higher mean age compared to persons with STK1 less than 2.0 pM, in accordance with the present study. In another health screening study (n=8,869) of persons working at an oil company, the number of persons with a STK1 level ≥2.0 pM was 5.8% (2), which was quit higher than found in the present study and the health screening performed in Changsha (Chen et al., 2008). The reason to the higher proportion of persons with STK1 value ≥ 2.0 pM is likely due to the working condition with oil pollutions and chemical exposures, that probably increases the risk of development of diseases, including malignancies. As found in the present study and the health screening study in Changsha (Chen et al., 2008), 85.2% of the persons had precancerous diseases and/or physiological changes related to development of malignancies (Zhang et al., 2010). One person with hepatoma was found. Furthermore, the number of workers with STK1 values ≥2.0 pM that were directly and long-term exposed to oil-related pollutants (drilling, transportations, geological prospecting) was significantly higher (7.8%, $p<0.05$), compared to persons responsible for pre-drilling works or services (3.9%). Thus, it seems that STK1 is a useful marker to predicting risk for development of disease, including malignancies, after exposure to petroleum chemicals (Blair et al., 2007). STK1 was also analyzed in 72 elderly persons participated in a limited health screening. Three persons were found to have elevated STK1 values (7.1, 7.0 and 1.8 pM). Pathology examination showed that these three persons had lung adenosquamous carcinoma, stomach cancer, esophageal squamous cell carcinoma, respectively (Zhang et al., 2008). In this respect, it should be mentioned that STK1 level is also affected by other transient physiological changes than changes related to malignancies. Thus, when judge the implication of an elevated STK1 value as a risk of development of malignancies, other reasons for the transient elevated STK1 values than potential malignancies should be taken in account (Topolcan et al., 2008). Therefore access to medical histories of patients is of importance.

In this study, and also in the two health screening studies of Changsha (Chen et al., 2008) and Jilin, (Zhang et al., 2010) a majority of persons with STK values ≥2.0 pM showed diseases linked to development of malignancies. However, the types of diseases in relation to malignancies found were different, probably depending on various occupation and life stile, such as office versus industry or city versus countryside. The frequency of hyperplasia breast and prostate were much higher in the city citizen group than in the oil industry group (Chen et al., 2008) while the frequencies of anemia and over-weight were higher among the oil company group of people (Zhang et al., 2010). As comparison, in a study on 18,073 women (20 ~ 69-year-old) living at the countryside of the east part of China (Duan, 2010), only 2.1% with breast hyperplasia was found.

The frequency of breast and prostate hyperplasia increases in city citizens of China and tends towards younger persons. The frequency of breast hyperplasia is now 30-50% of women with age around 40 years (Pang 2010) and 18% of prostate hyperplasia (Dingwei et al., 2007). About 8.8-21.8% of woman with breast hyperplasia show a higher risk of developing breast malignancy, especially among woman with adenoid gland disease and breast malignancy in their family history (Pang, 2010). With the social and economic progression in China, great changes have taken place in people's lifestyle such as early sexual maturity, delayed menopause, infertility, late childbearing, no breast-feeding, anxiety, bad mood, obesity, high calorie and high fat diet, unhealthy lifestyles, increased intake of exogenous estrogens (certain drugs, health products, cosmetics) and other environmental factors, and an increased incidence of breast cancer by 1-2% per year. Several studies proposal that multiple factors are involved in development of breast hyperplasia. Mammary stem cells have been recently identified. These stem cells may be drive oncogenic mutations. In normal stem cells, oncogenic mutations will allow

transit-amplifying cells to continue proliferating without entering a post-mitotic differentiated state (Cariati et al., 2008). Estrogens and progesterone affect the proliferative activity of breast epithelial cells, likely to have a role in the initiation of the cascade of events that leads breast malignancy (Im et al., 2009). Mutations in certain genes involved in the regulation of cell growth, such as p27 (Chan et al., 2010) and YB-1 dis (Yu et al., 2010) have been discussed. Other risk factors also include age, lifestyle and dietary components (Butler, et al., 2010).

The International Cancer Research Agency reported that the incidence rate of prostate cancer in China 2002 was 1.6/100,000, with a mortality rate of 1.0/100,000, rapidly increasing due to adaptation to West-lifestyle, and likely to exceed 10/100,000 in the near future (Dingwei et al., 2007). Prostatic intraepithelial neoplasia (PIN) and glandular intraepithelial neoplasia with high-grade (HGPIN) are considered as a precancerous lesion. The significance of prostate benign hyperplasia (PBH) and atypical adenomatous hyperplasia (AAH) for the development of prostate carcinoma is still debated, however, there could be a link to progression of malignancy. Shah et al (Shah et al., 2001) studied total 5,510 samples from patients with benign, simple atrophy (SA), prostatic hyperplasia (PAH), HGPIN and prostatic adenocarcinoma (PCA) tissues by Ki-67 microarray immunohistochemistry. They found that PAH was histologically distinct from SA, which has intermediate- to large-sized glands, minimal cytoplasm and inconspicuous nuclei, and a strong topographic association between PAH and PCA. Although HGPIN resembles PCA in its topographic distribution, cytological appearance, and molecular alterations including chromosome 8p loss, the chromosome 8c loss was significantly higher in PAH, and thus supporting its role as a neoplastic precursor. In another study approximately 3,000 neoplastic and benign prostate epithelial cells were isolated using laser capture microdissection from snap-frozen prostate biopsy specimens provided by 31 patients who subsequently participated in a clinical trial. Comparative analyses identified 954 transcript alterations associated with cancer (Qian et al., 2009). In a Chinese study, precancerous lesions were observed in prostate resected specimens, with an incidence rate of PIN of 56.8% and an incidence rate of AAH of 6.3% (Zhaozhan et al., 2002). Patients diagnosed with BPH and underwent transurethral resection of the prostate (TURP) or prostatectomy was found to have incidental prostatic carcinoma (IDPC) (Wang et al., 2002; Li et al., 2008; Wang et al., 2008). Thus, Li et al., (2008) reported that of 1,510 patients, 68 patients were found to have IDPC. In another two studies, the frequencies of IDPC were found to be 2.3% and 3.5%, respectively (Li et al. 2008, Wang et al. 2008). Recent data showed that prostate stem cell antigen (PSCA) mRNA expression in benign prostatic hyperplasia (BPH) patients with both PSA <4.0 ng/ml and normal DRE findings in TURP-resected tissues predicted subsequent prostate cancer (Zhao et al., 2009). In a study on 92 patients with prostate adenocarcinoma, the use of prostatic-specific antigen (PSA) and serological TK1 as markers for primary tumor, tumor spread to lymph node and metastatic disease was investigated (Letocha et al., 1996; O'Neill et al., 2001). Although the mean value of PSA of the metastatic group was significantly higher compared to the primary and lymph node groups, the individual values overlap extensively, making PSA not useful on individual patients. On the other hand, TK1 values in about 50% of the patients in the metastatic group were higher compared to the primary and lymph node groups and separated from patients in these two groups. Hence, TK1 in serum of prostate cancer patients seems to be more useful than PSA in discovering patients with metastatic prostate adenocarcinom (Letocha et al., 1996).

The frequency of woman with breast hyperplasia of moderate/severe type in this study was 38.3% in the Normal STK1 group, but increased significantly to 60.9% in the Elevated STK1 Group. A similar significantly increases was found in man with moderate/severe type of prostate hyperplasia (from 10,6% to 34,4%). No such increases were found among woman and man with mild hyperplasia. Thus, although we have not done a long time follow up the persons with elevated STK1 values and moderate/severe hyperplasia, the correlation between higher STK1 values and moderate/severe hyperplasia found in this study speaks in favor of that elevated STK1 values indicate a higher risk to develop at least breast and prostate malignancies. Furthermore, since STK1 values ≥ 2.0 pM are found in 65-90% of patients already have malignancies, and that persons with elevated STK1 values are significantly older than persons with low STK1 values (see Table 5, ref 1, 2), it strongly support that persons with elevated STK1 values show a higher risk to develop malignancies in the future. Thus, the STK1 seems to be a risk warning marker useful in health screening. However, follow up studies need to confirm that. Such studies are in progress. Preliminary results from such a follow up are given in this study.

In the present study and that of the health screening study from Chen et al., (2008), about 95% of the breast hyperplasia was of lobular type. These results are in consistent with an epidemiology investigation of breast cancer specimens of Chinese woman showing that about 83% of breast adenoids lobular hyperplasia (Hu, 2006). Lobular neoplasia broadly defines the spectrum of changes within the lobule, ranging from atypical lobular hyperplasia (ALH) to lobular carcinoma in situ (LCIS). These types of lesions are associated with an increased risk for developing subsequent invasive breast cancer, higher with proliferative changes (Bodian et al., 1996; Vogel, 2004; Anderson et al., 2006). This cancer risk is probably not constant over more than 15 years (Page et al., 1991). The presence of proliferative changes of atypic benign breast tissue should suggest breast-conservation therapy.

Obesity is now considered to be a multifunctional chronic disease, resulting from interactions between genetic and environmental factors (Nielsen, 2010). Obesity is a significantly increased risk factor of mortality in cancer and account for about 20% of some cancer types(52 Wolin et al., 2010). In an epidemiology investigation on 401,215 participants (Parr et al., 2010), an increased risk of mortality in cancer between 1.50 to 4.21 times was found, depending on type of malignancies. There was no difference between various geographic regions

(Asia, Australia and New Zeeland), apart from cancers of the oropharynx and larynx, where the association was inversed in Australia and New Zee land and absent in Asia. It is to be recommended obesity treatment and assessment guidelines for adults, adolescents, and children, multilevel prevention strategies and an approach to obesity management.

In summary, the present study indicates that STK1 is relevant for an early risk warning for development of malignancies, which are confirmed by recent health screening studies (Chen et al., 2008; Zhang et al., 2010). The slightly different number of persons with elevated STK1 values found between the three health screenings is probably due to different occupational groups, regions and lifestyles. Thus, it seems that STK1 can be used in health screening as a reliable tumor proliferating marker alone or in combination with routing inspections for an early risk warning assessment of malignant process. The STK1 assay can be applied to different occupations, particularly in cancer frequency high-risk areas, such as industries with chemical exposures, and among elderly populations. We believe that STK1 is a reliable marker for assessment tumor cell proliferation that can achieve the goal "tumor early detection and early treatment".

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