

Lower Blood Calcium Associates with Unfavorable Prognosis and Predicts for Bone Metastasis in NSCLC

Hongchang Shen^{1,2*}, Yongqiu Li^{3*}, Yida Liao^{1,2}, Tiehong Zhang^{1,4}, Qi Liu^{2*}, Jiajun Du^{2,4*}

1 Bio-bank Center, Provincial Hospital Affiliated to Shandong University, Shandong University, Jinan, People's Republic of China, **2** Institute of Oncology, Provincial Hospital Affiliated to Shandong University, Shandong University, Jinan, People's Republic of China, **3** Department of Medical Administration, Provincial Hospital Affiliated to Shandong University, Shandong University, Jinan, People's Republic of China, **4** Department of Thoracic Surgery, Provincial Hospital Affiliated to Shandong University, Shandong University, Jinan, People's Republic of China

Abstract

Ionized calcium was involved in various cellular signal pathways, and regulates many cellular processes, including those relevant to tumorigenesis. We hypothesized that imbalance of calcium homeostasis is correlated with development of lung carcinomas. We collected the clinical data of 1084 patients with non-small cell lung cancer (NSCLC) treated in Shandong Provincial Hospital, Shandong University. Logistic regression was used to determine the association between calcium levels and clinical characteristics, and COX regression and Kaplan-Meier model were applied to analyze risk factors on overall survival. Blood electrolytes were tested before treatment; and nearly 16% patients with NSCLC were complained with decreased blood calcium, which is more frequent than that in other electrolytes. Further, Multivariate logistic regression analysis disclosed that there were significant correlations between blood calcium decrease and moderate and poor differentiation ($P = 0.012$, $OR = 1.926$ (1.203–4.219)), squamous cell carcinoma ($P = 0.024$, $OR = 1.968$ (1.094–3.540)), and bone metastasis ($P = 0.032$, $OR = 0.396$ (0.235–0.669)). In multivariate COX regression analysis, advanced lymph node stage and decreased blood calcium were significantly and independently unfavorable prognostic factors ($P < 0.001$). Finally, the Kaplan-Meier Survival curve revealed that blood calcium decrease was associated with shorter survival (Log-rank; $\chi^2 = 26.172$, $P < 0.001$). Our findings indicate that lower blood calcium levels are associated with a higher risk of unfavorable prognosis and bone metastasis of NSCLC.

Citation: Shen H, Li Y, Liao Y, Zhang T, Liu Q, et al. (2012) Lower Blood Calcium Associates with Unfavorable Prognosis and Predicts for Bone Metastasis in NSCLC. PLoS ONE 7(3): e34264. doi:10.1371/journal.pone.0034264

Editor: Pan-Chyr Yang, National Taiwan University Hospital, Taiwan

Received: November 6, 2011; **Accepted:** February 24, 2012; **Published:** March 30, 2012

Copyright: © 2012 Shen et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by Provincial Natural Science Foundation of Shandong (ZR2010HM067 and ZR2011HM077) and Provincial Science and Technology Foundation of Shandong (2010RKG2007). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: liuqi66@sdu.edu.cn (QL); dujiajun@sdu.edu.cn (JD)

† These authors contributed equally to this work.

Introduction

Lung cancer is the most common malignancy all over the world and the leading cause of death in men [1]. A series of studies have investigated the relationship between calcium and lung cancer, especially cancer-related hypercalcaemia. However, few studies focus on association between the hypocalcaemia and lung cancer.

Calcium signal is a ubiquitous cellular signaling, which regulates cell death and proliferation by activating or inhibiting cellular signal pathways and calcium-regulated proteins [2]. Extracellular free calcium is about ~1.2 mM [3], and free calcium intercellular is 100 nM~1 mM. The aspects and degree in free calcium is associated with many pathways [4–7]. Thus, calcium regulates various cellular processes, including those relevant to tumorigenesis, such as cell motility, angiogenesis, gene transcription, apoptosis and proliferation. The intracellular calcium signaling is implicated in invasion [8,9], and extracellular calcium is associated with bone metastasis [10]. Calcium plays a key role in angiogenesis, such as VEGF can increase intracellular calcium by mobilizing calcium release from internal stores [11]; calcium is a modulator of poly (ADP-ribose) polymerase-1 (PARP1) activity [12]. Calcium signaling is implicated in the differentiation

process either through the extracellular calcium sensing receptor and/or alteration in intracellular calcium, which results in cancer cells to be dedifferentiated in the tumorigenic process [13]. Calcium is also involved in regulating cell cycle and apoptosis [14,4, and 5]. Reduction in the calcium content of the lumen of the endoplasmic reticulum is associated with resistance to apoptosis [5,15].

Currently, most studies about calcium focus on the relationship between calcium intake and the risk of aggressive or clinically relevant cancers [16–21]. Primary lung cancer complicated by hypercalcaemia was 26%. Hypercalcaemia may be due to the increased activity of osteoclasts causes bone calcium into the extracellular fluid caused by bone metastases; or caused by tumor secreted PTHrP. However, in our clinical practice, there were nearly 16% NSCLC patients with hypocalcaemia. The critical role of calcium in NSCLC progression remains uncertain. We hypothesized that lung carcinoma could break blood calcium homeostasis, whereas the imbalance of calcium will also have effects on the tumorigenesis and development of lung cancer.

To confirm the hypothesis, we collected the clinical data of lung cancer patients; tested the blood calcium levels; and followed up the patients. It is hopefully to find out the relationship between

Table 1. The changes of Blood electrolytes in 1084 cases with NSCLC.

Blood electrolytes	Calcium	Magnesium	Potassium	Sodium	Chlorine
Decreased	175	11	8	9	4
Normal	901	1064	1071	1068	1074
Increased [§]	8	9	5	7	6
<i>P</i>	<0.05 [#]				

NSCLC: non small cell lung cancer;

[§]These cases is not included, because it is too less and not enough for statistic analyses;

[#]the incidence of calcium decrease compare with others blood electrolytes, including magnesium, potassium, sodium and chlorine.

doi:10.1371/journal.pone.0034264.t001

blood calcium decrease and clinical staging, bone metastasis, differentiation and prognosis of lung carcinoma.

Results

Among the eligible 1084 patients, 175 cases were accompanied with blood calcium <2.2 μM , 11 cases magnesium decreased, 8 cases potassium decreased, 9 cases sodium decreased and 4 cases chlorine decreased. Data was shown in Table 1. The patients with blood electrolytes increased were not included, because the cases were too less to do statistic analysis. We found blood calcium decreased was more frequent than that in other electrolytes ($P < 0.05$) (Table 1).

Of 1084 accrued cases, 175 cases were followed with blood calcium <2.2 μM , 901 patients blood $2.2 \leq \text{calcium} \leq 2.6 \mu\text{M}$, and 8 cases calcium >2.6 μM . Patients' characteristics, grouped by calcium levels, were shown in Table 2. Among the 175 patients,

Table 2. Relations of clinical and pathological characteristics and Blood Calcium levels in NSCLC patients.

Characteristics	No. of patients			Chi-square	<i>P</i> value
	Calcium <2.2	$2.2 \leq \text{Calcium} \leq 2.6$	Calcium >2.6 [§]		
Sex					
Male	127	643	6	0.103	0.78
Female	48	258	2		
Age					
<60	63	410	3	5.374	0.024
≥ 60	112	491	5		
Smoking Index					
Never	53	351	2	5.539 ^a	0.019 ^a
<400	14	82	1		
≥ 400	108	467	5		
Tumor differentiation					
Well	11	231	1	32.308 ^b	0.000 ^b
Moderately	110	472	5		
Poorly	55	198	2		
Histology					
Adenomas	63	445	5	11.871 ^c	0.003 ^c
Squamous	101	394	3		
Others	11	62	0		
LN metastasis^{&}					
Negative	43	246	3	6.109	0.013
Positive	65	218	2		
Bone metastasis[▽]					
Negative	69	374	0	11.500	0.001
Positive	27	58	0		
Stage					
I	31	183	0	0.62	0.431
II-IV	144	718	8		

[§]These variables is not included, because it is too less and not enough for statistics analysis;

[&]This variable just included the resected NSCLC cases;

[▽]These cases came from the follow-up of resected NSCLC patients who were not detected bone metastases at primary care;

LN: Lymph node;

^aCompare of never smokers to smoking index ≥ 400 ;

^bCompare of well differentiation to moderately and poorly differentiation;

^cCompare of adenomas with squamous lung carcinomas, because others is too less and have special features.

doi:10.1371/journal.pone.0034264.t002

chi-square tests were used to evaluate the correlations between blood calcium levels and clinical and pathologic characteristics. There were no significant differences observed in gender ($\chi^2 = 0.103$, $P = 0.78$) and staging ($\chi^2 = 0.62$, $P = 0.431$). However, calcium decrease was significantly more prevalent in patients from age ≥ 60 than age < 60 ($P = 0.024$); from smokers than non-smokers ($P = 0.019$); in moderately and poorly differentiated tumors than in well differentiated tumors ($P < 0.001$); in squamous cell carcinoma than adenocarcinoma ($P = 0.003$); and in pN₀ tumors than in pN ≥ 1 tumors ($P = 0.013$). χ^2 tests showed that decreased baseline of calcium was a predictor for bone metastasis ($\chi^2 = 11.5$, $P < 0.001$) (**Table 2**).

Multivariate logistic regression analysis for the association of calcium levels with various characteristics disclosed that there were significant correlation between calcium decrease and moderate and poor differentiation ($P = 0.012$, OR = 1.926 (1.203–4.219)), squamous cell carcinoma ($P = 0.024$, OR = 1.968(1.094–3.540)), and bone metastasis ($P = 0.032$, OR = 0.396(0.235–0.669)) (**Table 3**).

Further, chi-square tests were applied to analyze correlation between univariate analyses of clinical and pathologic characteristics and overall survival in 572 patients who were eligible for survival analysis (see the material and methods). As shown in Table 4, gender, tumor differentiation, lymph node metastasis, p-staging and blood calcium were significantly correlated with prognosis ($P < 0.05$) (**Table 4**). In multivariate Cox Regression analysis, only advanced lymph node stage and decreased blood calcium were significantly and independently unfavorable prognostic factors ($P < 0.001$) (**Table 5**).

Finally, we analyzed the effects of blood calcium decrease on survival using the Kaplan-Meier Survival Model in 572 patients who underwent radical resection, including 108 cases with blood calcium $< 2.2 \mu\text{M}$ and 464 cases with $2.2 \leq \text{calcium} \leq 2.6 \mu\text{M}$ (**Fig. 1**). Log-rank comparisons revealed that blood calcium decrease ($< 2.2 \mu\text{M}$) was associated with shorter survival (Log-rank; $\chi^2 = 26.172$, $P < 0.001$).

Discussion

In current study, the integrated and advanced bio-bank was used to examine association between blood electrolytes and NSCLC. We found decreased blood calcium correlated with poor prognosis, poor tumor differentiation, and squamous cell carcinoma. We also found that decreased blood calcium at initial care may be a predictor for bone metastasis. In previous studies, blood calcium levels correlated with aggressive cancers have generated mixed results [16–19,22–24]. Many studies have shown that a high intake of calcium may increase the risk of prostate cancer, and the possible reason is high dietary intake of calcium would lead to lower vitamin D which is an established risk factor for prostate cancer [25]. However, there were also studies found no strong association between dietary calcium intake and risk of prostate cancer [26]. The role of calcium in tumorigenesis is unclear. Our study may be the first focus on the correlation between decreased blood calcium and clinical characteristics and prognosis of NSCLC.

As mentioned above, we believe the ionized calcium plays an important role in lung cancer development. Blood electrolytes were tested before treatments; and we found nearly 16% patients with NSCLC were complained with decreased blood calcium,

Table 3. Multivariate Logistic Regression Analysis for the correlation between calcium levels and clinical characteristics.

Variable*	Adjusted OR (95%CI)	P Value
Age		
<60	0.720(0.433–1.199)	0.207
≥ 60		
Smoking Index		
Never	1.246(0.657–2.363)	0.50
≥ 400		
Tumor differentiation		
Well	1.926(1.203–4.219)	0.012
Moderately and poorly		
Histology		
Adenomas	1.968(1.094–3.540)	0.024
Squamous		
LN metastasis^{&}		
Negative	0.714(0.427–1.195)	0.20
Positive		
Bone metastasis[▽]		
Negative	0.396(0.235–0.669)	0.032
Positive		

*These variables were significantly correlated with Blood Calcium, which were shown in Table 2;

[&]This variable just included the resected NSCLC cases;

[▽]These cases came from the follow-up of resected NSCLC patients who were not detected bone metastases at primary care;

OR: Odds Ratio; CI: confidence Interval; LN: Lymph node.

doi:10.1371/journal.pone.0034264.t003

Table 4. Univariate analysis of clinical characteristics' affect on 3 years survival.

Characteristics	Chi-square	P value
Sex		
Female vs Male	6.783	0.009
Age		
<60 vs ≥ 60	1.374	0.088
Smoking Index		
Never vs ≥ 400	0.935	0.241
Tumor differentiation		
Well vs Moderately and Poorly	23.803	<0.01
Histology		
Adenomas vs Squamous	0.781	0.356
LN metastasis^{&}		
Negative vs Positive	16.109	0.003
Bone metastasis[▽]		
Negative vs Positive	1.047	0.158
Stage		
I vs II–IV	10.744	0.006
Calcium		
<2.2 vs $\geq 2.2, \leq 2.6$	8.462	0.027

[&]This variable just included the resected NSCLC cases;

[▽]These cases came from the follow-up of resected NSCLC patients who were not detected bone metastases at primary care;

LN: Lymph node.

doi:10.1371/journal.pone.0034264.t004

Table 5. Multivariate analysis of clinical characteristics affect on 3-year survival using COX regression model.

Variables [△]	HR(95%CI)	P value
Sex		
Female vs Male	1.034(0.722–1.481)	0.856
Tumor differentiation		
Well vs Moderately and Poorly	1.348(0.547–3.324)	0.516
LN metastasis		
Negative vs Positive	2.237(1.469–3.406)	0.000
Stage		
I vs II–IV	0.742(0.511–1.077)	0.117
Calcium		
<2.2 vs ≥2.6	2.697(1.590–4.574)	0.000

[△]These variables were selected from the variables that have effects on 3-year survival using univariate analysis;
HR: Hazard Ratio; CI: Confidence Interval; LN: Lymph node.
doi:10.1371/journal.pone.0034264.t005

which was a rather frequent event. Based on our research, it is reasonable to speculate that tumor differentiation and clinical staging might modify the relationship between blood calcium levels and NSCLC risk.

It has been shown that calcium level played a role in the development and progression of breast and prostate cancers [27,28]. A high level of serum calcium is associated with aggressive, poorly differentiated lesions or fatal prostate cancer, which means calcium homeostasis is important to keep body in normal [29,30]. The broken of calcium homeostasis will promote lung cancer development, which is very harmful to NSCLC patients. Given the broad range of biological functions dependent on calcium, it is highly plausible that calcium deficiency may affect multiple pathways in tumorigenesis. For instance, a recent study found extracellular calcium may function to regulate the

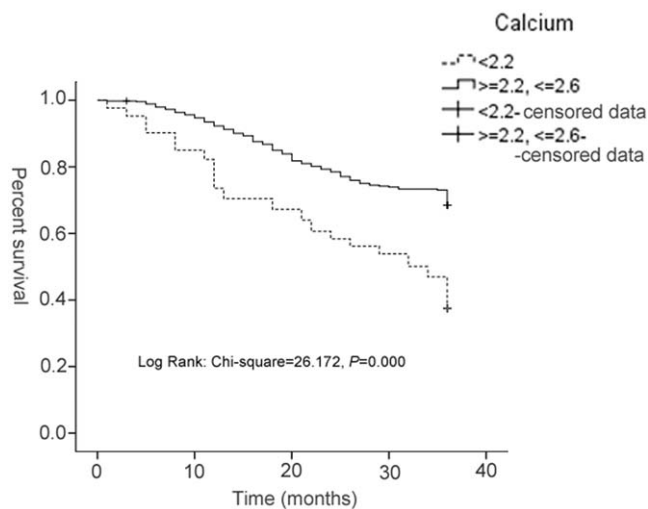


Figure 1. The Kaplan-Meier survival curve of NSCLC patients who underwent radical resection (n = 572). It is analyzed using Kaplan-Meier survival model, including 108 cases with blood calcium <math>< 2.2</math>, and 464 cases with $2.2 \leq \text{calcium} \leq 2.6$. Log-rank comparisons revealed that blood calcium decrease (<math>< 2.2 \mu\text{M}</math>) was associated with shorter survival (Log-rank; $\chi^2 = 26.172, P < 0.001$).
doi:10.1371/journal.pone.0034264.g001

differentiation of colonic epithelial cells and may contribute to abnormal differentiation and malignant progression [31]. Inter-cellular calcium strongly affected by extracellular calcium regulates prostate cancer cell growth and results in a greater proportion of unaggressive tumors [32], which is consistent to our finding.

To confirm the association between decreased blood calcium and NSCLC bone metastasis, we tested the blood calcium among the patients without bone metastasis; and followed up them at least 36 months for the alives. We found decreased calcium is strongly associated with bone metastasis compare with calcium in normal. The possible reasons are that hypocalcaemia stimulates secretion of parathyroid hormone to increase blood calcium and enhance osteolytic cells to break bone, resulting in osteoporosis, which provides opportunity for cancer cells invasive the bones. This view need more evidences to confirm, and we will do further studies to clear the mechanisms.

Our study at least to some degree provides one possible evidences for some inconsistency in the previous studies. Our finding indicates that lower blood calcium levels are associated with a higher risk of unfavorable prognosis and bone metastasis of NSCLC. These findings, if confirmed, may provide a new way for the personalized adjuvant care and novel predictor for bone metastasis of NSCLC.

Materials and Methods

Patients

The study was approved by the Ethical Committee of Shandong Provincial Hospital affiliated to Shandong University. All patients' records were from the Bio-Bank of Shandong Provincial Hospital. A retrospective cohort design was applied to assess and identify all pathologically confirmed, newly diagnosed cases of primary non small cell lung cancer during January 2006 and June 2008. All patients received a primary care, and most patients obtained their longitudinal care in Shandong Provincial Hospital. Criteria for eligibility included Karnofsky performance score ≥ 70 , weight loss $\leq 5\%$ in the prior 3 months, $18 \leq \text{age} \leq 75$, blood electrolytes assay at first visit and not other chronic diseases such as diabetes mellitus and hypertension. There were 1267 patients newly diagnosed as lung cancer during January 2006 and June 2008, and 1084 cases were eligible, including 572 resected and 512 non-resected NSCLC. The level of blood electrolytes was tested before primary treatment. Overall survival was analyzed in 572 patients: a. survived for >3 months after surgery, b. followed up at least 3 years after surgery for the alives, c. did not die of any other reasons excluding lung cancer. Written informed consent was obtained from all participating subjects.

To ensure reliable and consistent data collection, two independent trained physicians who were supervised by the study team were assigned to review the relevant database of each case. Otherwise all conflicts were discussed to obtain agreement. After the review, we excluded patients who died in one month from primary care; death reasons were not malignancy and whose pathologic diagnoses were not done in Shandong Provincial Hospital.

Statistical Analysis

The relations between blood calcium levels and clinical and pathological characteristics were analyzed by chi-square tests or Fisher exact tests. Multivariate logistic regression analysis was applied to estimate the factors on blood calcium. Chi-square tests were used to evaluate correlations between single variable and overall survival. The correlations between multiple variables and

3-year survival were measured by multivariate Cox regression model. Variables entered into the multivariate COX regression models were owed from the variables that were statistically significant at the 0.1 level in univariate analysis. The Kaplan-Meier survival curves were applied to assess the effects of blood calcium on 3-year Survival, and the log-rank test was used to evaluate the differences in survival distributions. $P < 0.05$ was set as

statistical significance. All the statistical analyses were completed by SPSS software (version 17.0).

Author Contributions

Conceived and designed the experiments: HS JD QL. Performed the experiments: HS Y. Liao TZ. Analyzed the data: Y. Li. Contributed reagents/materials/analysis tools: Y. Li. Wrote the paper: HS JD.

References

- Jemal A, Siegel R, Ward E, Hao Y, Xu J, et al. (2009) Cancer statistics, 2009. *CA Cancer J Clin* 59: 225–249.
- Berridge MJ, Bootman MD, Roderick HL (2003) Calcium signalling: dynamics, homeostasis and remodelling. *Nature Reviews Molecular Cell Biology* 4: 517–529.
- Golovina VA, Blaustein MP (1997) Spatially and functionally distinct Ca²⁺ stores in sarcoplasmic and endoplasmic reticulum. *Science* 275: 1643–1648.
- Rizzuto R, Pozzan T (2006) Microdomains of intracellular Ca²⁺: molecular determinants and functional consequences. *Physiol Rev* 86: 369–408.
- Rosario Rizzuto, Paolo Pinton, Davide Ferrari, Mounia Chami, György Szabadkai, et al. (2003) Calcium and apoptosis: facts and hypotheses. *Oncogene* 22: 8619–8627.
- Berridge MJ (1997) The AM and FM of calcium signalling. *Nature* 386: 759–760.
- Lipskaia L, Lompre AM (2004) Alteration in temporal kinetics of Ca²⁺ signaling and control of growth and proliferation. *Biol Cell* 96: 55–68.
- Govindasamy A, Gopa B, Hindupur K, Camasamudram V, Henry MS, et al. (2002) Mitochondrial stress-induced calcium signaling, phenotypic changes and invasive behavior in human lung carcinoma A549 cells. *Oncogene* 21: 7839–7849.
- Huang JB, Kindzelskii AL, Clark AJ, Petty HR (2004) Identification of channels promoting calcium spikes and waves in HT1080 tumor cells: their apparent roles in cell motility and invasion. *Cancer Res* 64: 2482–2489.
- Liao J, Schneider A, Datta NS, McCauley LK (2006) Extracellular calcium as a candidate mediator of prostate cancer skeletal metastasis. *Cancer Res* 66: 9065–9073.
- Patton AM, Kassir J, Doong H, Kohn EC (2003) Calcium as a molecular target in angiogenesis. *Curr Pharm Des* 9: 543–551.
- Bentle MS, Reinicke KE, Bey EA, Spitz DR, Boothman DA (2006) Calcium-dependent modulation of poly (ADP-ribose) polymerase-1 alters cellular metabolism and DNA repair. *J Biol Chem* 281: 33684–33696.
- Bikle DD, Oda Y, Xie Z (2004) Calcium and 1,25(OH)₂D: interacting drivers of epidermal differentiation. *J Steroid Biochem Mol Biol* 89–90: 355–360.
- Cullen PJ, Lockyer PJ (2002) Integration of calcium and Ras signalling. *Nature Rev. Mol Cell Biol* 3: 339–348.
- Pinton P, Rizzuto R (2006) Bcl-2 and Ca²⁺ homeostasis in the endoplasmic reticulum. *Cell Death Differ* 13: 1409–1418.
- Ahn J, Albanes D, Peters U, Schatzkin A, Lim U, et al. (2007) Dairy products, calcium intake, and risk of prostate cancer in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev* 16: 2623–2630.
- Halthur C, Johansson AL, Almquist M, Malm J, Gronberg H, et al. (2009) Serum calcium and the risk of prostate cancer. *Cancer Causes Control* 20: 1205–1214.
- Severi G, English DR, Hopper JL, Giles GG (2006) Re: Prospective studies of dairy product and calcium intakes and prostate cancer risk: a meta-analysis. *J Natl Cancer Inst* 98: 794–795.
- Giovannucci E, Liu Y, Stampfer MJ, Willett WC (2006) A prospective study of calcium intake and incident and fatal prostate cancer. *Cancer Epidemiol Biomarkers Prev* 15: 203–210.
- Kesse E, Bertrais S, Astorg P, Jaouen A, Arnault N, et al. (2006) Dairy products, calcium and phosphorus intake, and the risk of prostate cancer: results of the French prospective SU.VI.MAX (Supplementation en Vitamines et Minéraux Antioxydants) study. *Br J Nutr* 95: 539–545.
- Rodriguez C, McCullough ML, Mondul AM, Jacobs EJ, Fakhraabadi-Shokoochi D, et al. (2003) Calcium, dairy products, and risk of prostate cancer in a prospective cohort of United States men. *Cancer Epidemiol Biomarkers Prev* 12: 597–603.
- Gao X, LaValley MP, Tucker KL (2005) Prospective studies of dairy product and calcium intakes and prostate cancer risk: a meta-analysis. *J Natl Cancer Inst* 97: 1768–1777.
- Baron JA, Beach M, Wallace K, Grau MV, Sandler RS, et al. (2005) Risk of prostate cancer in a randomized clinical trial of calcium supplementation. *Cancer Epidemiol Biomarkers Prev* 14: 586–589.
- Halthur C, Johansson AL, Almquist M, Malm J, Gronberg H, et al. (2009) Serum calcium and the risk of prostate cancer. *Cancer Causes Control* 20: 1205–1214.
- Giovannucci E (1998) Dietary influences of 1,25(OH)₂ vitamin D in relation to prostate cancer: a hypothesis. *Cancer Causes Control* 9: 567–582.
- Ahn J, Albanes D, Peters U, Schatzkin A, Lim U, et al. (2007) Dairy products, calcium intake, and risk of prostate cancer in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev* 16: 2623–2630.
- Almquist M, Manjer J, Bondeson L, Bondeson AG (2007) Serum calcium and breast cancer risk: results from a prospective cohort study of 7,847 women. *Cancer Causes Control* 18: 595–602.
- Prins GS, Korach KS (2008) The role of estrogens and estrogen receptors in normal prostate growth and disease. *Steroids* 73: 233–244.
- Skinner HG, Schwartz GG (2009) A prospective study of total and ionized serum calcium and fatal prostate cancer. *Cancer Epidemiol Biomarkers Prev* 18: 575–578.
- Skinner HG, Schwartz GG (2008) Serum calcium and incident and fatal prostate cancer in the National Health and Nutrition Examination Survey. *Cancer Epidemiol Biomarkers Prev* 17: 2302–2305.
- Subhas C, Venugopal R, Henry A, James V (2003) Extracellular Calcium and Calcium Sensing Receptor Function in Human Colon Carcinomas. *Cancer research* 63(1): 67.
- Guillaume L, Sandrine H, Christian S, Etienne D, Fabien VA, et al. (2001) Ca²⁺ Pools and Cell Growth. *Journal of Biological Chemistry* 276: 47608.