Identification of genes down-regulated during lung cancer progression: A cDNA array study

Mara Campioni\textsuperscript{1}, Vincenzo Ambrogi\textsuperscript{2}, Eugenio Pompeo\textsuperscript{2}, Gennaro Citro\textsuperscript{3}, Mauro Castelli\textsuperscript{4}, Enrico P Spugnini\textsuperscript{3}, Antonio Gatti\textsuperscript{2}, Pierluigi Cardelli\textsuperscript{3}, Laura Lorenzon\textsuperscript{5}, Alfonso Baldi*\textsuperscript{1} and Tommaso C Mineo\textsuperscript{2}

Address: \textsuperscript{1}Department of Biochemistry and Biophysics "F. Cedrangolo", Section of Pathology, Second University of Naples, Naples, Italy, \textsuperscript{2}Department of Thoracic Surgery and Department of Anaesthesiology, Tor Vergata University, Rome, Italy, \textsuperscript{3}S.A.F.U. Department, CRS, "Regina Elena" Cancer Institute, Rome, Italy, \textsuperscript{4}Experimental Oncology Department, "Regina Elena" Cancer Institute, Rome, Italy and \textsuperscript{5}Department of Surgery "A", Second Faculty of Medicine, "La Sapienza" University, Rome, Italy

Email: Mara Campioni - maracampioni@yahoo.it; Vincenzo Ambrogi - vambrogi@libero.it; Eugenio Pompeo - pompeo@uniroma2.it; Gennaro Citro - Citro@ifo.it; Mauro Castelli - castelli@ifo.it; Enrico P Spugnini - spugnini.vet@tiscali.it; Antonio Gatti - gatti@uniroma2.it; Pierluigi Cardelli - card@hotmail.com; Laura Lorenzon - doc_lauretta@hotmail.com; Alfonso Baldi* - alfonso.baldi@tiscali.it; Tommaso C Mineo - mineo@uniroma2.it

\* Corresponding author

Abstract

**Background:** Lung cancer remains a major health challenge in the world. Survival for patients with stage I disease ranges between 40–70\%. This suggests that a significant proportion of patients with stage I NSCLC may actually be under-staged.

**Methods:** In order to identify genes relevant for lung cancer development, we carried out cDNA array experiments employing 64 consecutive patients (58 men and 6 women) with a median age of 58 years and stage I or stage 2 non-small-cell lung cancer (NSCLC).

**Results:** Basic cDNA array data identified 14 genes as differentially regulated in the two groups. Quantitative RT-PCR analysis confirmed an effective different transcriptional regulation of 8 out of 14 genes analyzed. The products of these genes belong to different functional protein types, such as extra-cellular matrix proteins and proteases (Decorin and MMP11), genes involved in DNA repair (XRCC1), regulator of angiogenesis (VEGF), cell cycle regulators (Cyclin D1) and tumor-suppressor genes (Semaphorin 3B, WNT-5A and retinoblastoma-related Rb2/p130). Some previously described differences in expression patterns were confirmed by our array data. In addition, we identified and validated for the first time the reduced expression level of some genes during lung cancer progression.

**Conclusion:** Comparative hybridization by means of cDNA arrays assisted in identifying a series of novel progression-associated changes in gene expression, confirming, at the same time, a number of previously described results.
**Introduction**

Lung cancer remains a major health challenge in the world. Despite improvements in staging and the integrated application of surgery, radiotherapy, and chemotherapy, the 5-year survival rate for individuals with lung cancer is only about 15% [1]. Histologically, 80% of the lung cancers are diagnosed as non-small-cell lung cancer (NSCLC), whereas the remaining 20% of cases are diagnosed as small-cell lung cancer (SCLC). On the basis of cell morphology, adenocarcinoma and squamous cell carcinoma are the most common types of NSCLC. The current staging system for NSCLC is based upon the size and location of the primary tumor (T), the involvement of regional lymph nodes (N), and the presence of distant metastases (M) [1]. The standard treatment of patients with stage I NSCLC (T1-2, N0, M0) is resection of the primary tumor alone (no adjuvant therapy) [2]. Survival for patients with stage I disease ranges between 40–70%, and the failure is due to distant recurrences [3]. This suggests that a significant proportion of patients with stage I NSCLC may actually be under-staged. Therefore, if correctly identified, these patients may benefit from adjuvant therapy in addition to resection, with a predictable improvement in the survival rates. Indeed, to identify patients with stage I NSCLC who might benefit from adjuvant therapy, investigators have attempted to identify factors predicting poor prognosis. These studies included analysis of performance status, histologic subtype, size of the primary tumor, the degree of tumor differentiation, mitotic rate, and evidence of lymphatic or vascular invasion [4-8]. However, all of these factors have failed, to date, to precisely identify a group of stage I patients who would benefit from adjuvant therapy. Cigarette smoking remains the main risk factor for lung cancer, accounting for about 90% of the cases in men and 70% of the cases in women [9]. Our research group has investigated in the last years the possible involvement of several molecular mechanisms, such as cell cycle and apoptosis regulators, oncoproteins and tumor suppressor genes, cell adhesion molecules, in the pathogenesis and progression of lung cancer [10-19].

In this study, we utilized the cDNA array technique to identify genes differently expressed in patients at early stage of NSCLC.

**Materials and methods**

**Patient selection**

Table 1 summarizes the characteristics of the patients enrolled in the study. Subjects selected for the analysis were 64 patients, consecutively treated at the Department of Thoracic Surgery of the Tor Vergata University in Rome, who had radical surgery for NSCLC at pathological stage 1 or 2. The study project was submitted and approved by the Human Tissue Use Committee of the University. The patients were staged according to operative and pathological findings based on AJCC/UICC-TNM classification and stage grouping [17]. N-factor was assessed on lymph nodes removed during routine mediastinal lymphadenectomy. A preoperative staging computed tomography (CT) scan was performed in all patients. Histology grading and N-stage were performed on haematoxylin-eosin stained sections. In 22 patients the neoplasm was resected by pneumonectomy and in 42 by lobectomy. Patients who did not survive beyond 60 days after surgery were not included in the study to avoid bias from peri-operative death. Patients who underwent minimal resection were ruled out from the present analysis. Another mandatory prerequisite, was the lack of chemo or radiotherapy before and after surgical resection.

**RNA extraction**

Total RNA from frozen tumour tissues was extracted utilizing the Atlas™ Pure Total RNA Extraction System (Clontech). RNAs were quantified spectrophotometrically and their integrity confirmed by fractionation of 1 μg of RNA on 1% agarose gel with ethidium bromide staining. Then, two populations of RNAs were prepared, including exactly 1 μg of RNA from each patients, separating patients in stage 1 and stage 2.

**Array hybridization**

We prepared cRNA for hybridization using the Atlas™ Pure Total RNA labeling System (Clontech). The Clontech Atlas™ Human Cancer Array, composed of 588 human cDNAs, was used for hybridization according to the manufacturer’s instructions. Arrays were scanned using a PhosphorImager and analyzed by ImageQuant 5.0 software (Molecular Dynamics, Sunnyvale, California). The experiment was performed in duplicate, utilizing two different RNA preparations. Intensity values were normalized using the expression levels of the housekeeping genes spotted.

| Table 1: Patients’ Characteristics |
|-----------------------------------|
| **Total number**                  | 64 |
| **Median age**                    | 58 |
| **Male vs Female**                | 58 vs 6 (90.5% vs 9.5%) |
| **Neoplasm histotype**            |    |
| Squamous cell carcinoma           | 33 (51.5%) |
| Adenocarcinoma                    | 23 (36%) |
| Others                            | 8 (12.5%) |
| **Clinical Stage**                |    |
| I                                 | 42 (65.5%) |
| II                                | 22 (34.5%) |
| **Grading**                       |    |
| I–2                               | 38 (59.5%) |
| 3                                 | 26 (40.5%) |
| **Surgery**                       |    |
| Pneumonectomy                     | 22 (34.5%) |
| Lobectomy                         | 42 (65.5%) |
on the arrays. A cut-off of two folds was used to select the genes differentially expressed.

Quantitative real-time reverse transcription-PCR

The primer sequences are shown in table 2. Primers were designed using Primer Express 2.0 software (Applied Biosystem, Foster City, CA, USA). The specificity of each target amplicon was assessed by dissociation curve analysis and all amplicons were spanning over exon-exon regions to avoid genomic amplification. qPCR was performed on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, CA, USA) in 384 well plates assembled by Biorobot 8000 (Qiagen, Hilden, Germany) using a final volume of 20 μl and the following cycle conditions: 50°C for 2 min., 95°C for 10 min., and then 40 cycles of 15 s at 95°C and 1 min at 60°C. All QPCR mixtures contained 1 μl of cDNA template (corresponding to 20 ng retro-transcribed totRNA), 1× Sybr Green PCR Master Mix (2×) (Applied Biosystems, CA, USA) and 150 μM of each target-specific primer. For each experiment a no-template reaction was included as negative control. Results have been analyzed using the Applied Biosystems analysis software and expression levels calculated from a linear regression of the standard curve. Results are given as gene target expression vs GADPH expression (gene target relative expression) to correct for differences in the quantity of cDNA used in the PCR reaction. All real-time PCR reactions for each sample were performed in triplicate.

Results

A typical representation of the results obtained hybridizing two identical filter arrays with cDNA-labeled probes generated from either patients in stage 1 and patients in stage 2 is shown in Figure 1, Panels A and B. Computer-assisted analysis of these filters allowed, after normalization, to identify 14 genes, out of 588, as differentially regulated in the two groups (data not shown). To confirm these changes in expression patterns, the genes were further analyzed by Quantitative RT-PCR, using two sets of independently prepared RNAs. Only 8 out of 14 of the genes analyzed were confirmed to be differentially regulated by quantitative RT-PCR. In table 2 are depicted the genes whose differential expression was confirmed with quantitative RT-PCR. In table 3 the primers utilized for RT-PCR are shown. These genes code for different protein families: extra-cellular matrix proteins and proteases (Decorin and MMP11), genes involved in DNA repair (XRCC1), regulator of angiogenesis (VEGF), cell cycle regulators (Cyclin D1) and tumor-suppressor genes (Semaphorin 3B, WNT-5A and retinoblastoma-related Rb2/p130).

Discussion

In the present study, we analyzed two population of NSCLC patients at stage 1 and 2 to screen for tumor progression-associated genes, by means of a cDNA array filter containing 588 different cDNAs. Consistent with previously reported results [20], not all of the differences found by the array were effectively reproducible using another quantitative mRNA technique, such as quantitative RT-PCR. The discrepancies could be explained by methodological limitations of the array technology, where thousands of diverse cDNAs differing exponentially in expression levels are compared by means of a single hybridization reaction, irrespective of the optimal range of reaction conditions.

The genes we found reliably differentially regulated in the two RNA populations are known to exert different functions, thus confirming that the biological events taking place during the malignant progression involve several molecular pathways.

The cyclin D1 gene codes for one of the cyclins involved in cell cycle regulation; specifically for the G1-S transition. The involvement of cell-cycle regulators in the progression of lung cancer is well documented [21]. This observation, therefore, further support the fact that alterations in cell cycle regulation is one of the earlier steps in the progression of NSCLC.

The VEGF gene codes for a glycoprotein with potent angiogenic, mitogenic, and vascular permeability-enhancing activities for endothelial cells. Tumour neoangiogenesis has been recently recognized to be of importance in defining subsets of patients with poor outcome in cancer.

Table 2: Expression patterns obtained by array hybridization and confirmation of differential gene expression by Quantitative RT-PCR

| Gene         | Ref seq ID | Array ratio Stage 1/stage 2 | Confirmed by QPCR |
|--------------|------------|----------------------------|-------------------|
| Cyclin D1    | NM_053056  | 2.04 down                  | Yes               |
| VEGF         | NM_003376  | 2.02 up                    | Yes               |
| WNT-5A       | NM_003392  | 2.03 down                  | Yes               |
| MMP11        | NM_005940  | 3.68 up                    | Yes               |
| Rb2/p130     | NM_005611  | 3.24 down                  | Yes               |
| Semaphorin   | NM_004263  | 3.43 down                  | Yes               |
| Decorin      | NM_001920  | 2.16 up                    | Yes               |
| XRCC1        | NM_006297  | 3.40 down                  | Yes               |
From that several reports [18,24] have stated that the presence of neoangiogenesis represents a significant factor in terms of overall and disease free survival in lung cancer.

The WNT-5A gene is a representative ligand that activates a beta-catenin-independent pathway in the Wnt signalling [25]. In contrast to the transforming members of the Wnt family, shown to be up-regulated in many cancers, the role of Wnt 5a is still controversial. While it has been attributed a tumour suppressor function in some malignancies, there is increasing evidence of promigratory and proinvasive effects in others, mediated predominantly through the planar cell polarity pathway and activation of protein kinase C [26,27]. In our experimental setting, transcription of WNT-5A was down-regulated in stage 2.

**Table 3: Primers and conditions utilized for the Quantitative RT-PCR**

| Gene       | Sequence                     | PCR product (bp) | Annealing Temp. |
|------------|------------------------------|------------------|-----------------|
| Cyc D1 for | TGAGCCGGTAGTGAAAGCACAGG      | 66               | 60°C            |
| Cyc D1 rev | GACCTCCTCGTGCCTCTG          |                  |                 |
| VEGF for   | GCAGTAGCTGCGCTGATAGA         | 119              | 60°C            |
| VEGF rev   | CCTGCTGCTGCTACCTCCAC         |                  |                 |
| WNT-5A for | CAAAGCAACTCCGTGGCCTTA        | 107              | 60°C            |
| WNT-5A rev | GCTCGGATTCCTCGGCT            |                  |                 |
| MMP11 for  | CAGCCAAAGAGAATGCGGAGACC     | 147              | 60°C            |
| MMP11 rev  | GATGCCCTCTGAGATGC           |                  |                 |
| Rb2/p130 for | AGGAGTTTTCTCTGTGCAGTATCG   | 106              | 60°C            |
| Rb2/p130 rev | TGAACACTCTGCTCCTC          |                  |                 |
| Semaphorin for | TCACAGTGCCAGCATAGAGG     | 111              | 60°C            |
| Semaphorin rev | TCACAGTGCCAGCATAGAGG       |                  |                 |
| Decorin for | TGCAGGTCTAGCAGATGGTGTG     | 91               | 60°C            |
| Decorin rev | AATGCCACATCTCGAGTGGTC        |                  |                 |
| XRCCI for  | GACACTTACGAAATGGCC          | 110              | 60°C            |
| XRCCI rev  | GACACTTACGAAATGGCC          |                  |                 |
NSCLC, thus underlying a putative tumour suppressor function of its gene product in lung cancer progression.

The MMP11 gene belongs to a family of matrix metalloproteinases, proteolytic enzymes that degrade extracellular matrix and promote the local or metastatic potential of carcinoma cells, and whose action is restrained by special inhibitors (metalloproteinase inhibitors) [28,29]. The role of MMP11 protease in lung cancer progression has been clearly defined [28,29].

The Rb2/p130 gene codes for one of the retinoblastoma-related proteins. Its role as tumour-suppressor is well documented [30,31], as well as its involvement in lung cancer progression [14]. Our data further suggest that alteration on Rb2/p130 gene expression is involved also in the initial steps of NSCLC progression.

Loss of Semaphorin-A3F genes occurs frequently in lung cancer and correlates with advanced stage of disease [32]. Moreover, in lung cancer patients, semaphoring gene loss correlates with advanced disease and increased VEGF binding to tumour cells [33,34]. Our data confirms this phenomenon and correlates it also to the initial step of NSCLC progression.

The Decorin gene codes for one of the major extracellular matrix protein which has become the focus of various cancer studies [35]. The exact biological role of decorin in cancer has yet to be clarified; however, several experimental data suggest its involvement in cancer progression and metastatization [36].

The XRCCI gene codes for a base excision repair. Several papers have investigated the association between lung cancer and genetic polymorphisms in XRCCI, suggesting a predisposition to lung cancer development for the patients carrying these gene alterations [37,38]. Interestingly, it has been recently described that XRCCI transcript abundance levels correlate with cisplatin chemoresistance in non-small cell lung cancer cell lines [39]. To the best of our knowledge, no previous report exists about the involvement of the XRCCI gene in the early steps of NSCLC progression.

Conclusion

This study confirms the aptitude of the cDNA array technology in defining molecular pathways involved in NSCLC progression. In addition, our results corroborated previously observed expression patterns of a series of genes, and revealed new genes differentially expressed during NSCLC progression. The role of these newly identified genes is being evaluated in further studies analyzing protein expression pattern and function of the proteins in vitro and in vivo in lung cancer cells.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors read and approved the final manuscript. MC set up the cDNA array protocols and performed the experiment; VA, EP and AG collected the cancer samples; GC and MC gave advise on the work and helped in the interpretation of the data; EP, PC and LL performed the RNA extractions; AB supervised all the work and wrote the paper together with TCM.

Acknowledgements

This work was supported by grants from Ministry of Health, MIUR, Second University of Naples and FUTURA-onlus to A.B.; by FIRB/MUR (RBIP06LC9-009) to G.C.; by the Italian Health Ministry (Title of the project: "Profilo genetico associato al fenotipo metastatico e alla prognosi nei tumori polmonari") to TCM.

References

1. Zochbauer-Muller S, Gazdar AF, Minna JD: Molecular pathogenesis of lung cancer. Annu Rev Physiol 2002, 64:681-708.
2. D’Amico TA, Aloia TA, Moore MB, Conlon DH, Herndon JE 2nd, Kinch MS, Harpole DH Jr: Predicting the sites of metastases from lung cancer using molecular biologic markers. Ann Thorac Surg 2001, 72:1144-1148.
3. Harpole DH Jr, Herndon JE 2nd, Young WG Jr, Wolfe WG, Sabiston DC Jr: Stage I non small cell lung cancer. A multivariate analysis of treatment methods and patterns of recurrence. Cancer 1995, 76:787-796.
4. Feld R, Rubinstein LV, Weisenberger TH: Sites of recurrence in resected stage I non-small-cell lung cancer: a guide for future studies. J Clin Oncol 1984, 2:1352-1358.
5. D’Amico TA, Massey M, Herndon JE 2nd, Moore MB, Harpole DH Jr: A biologic risk model for stage I lung cancer: immunohistochemical analysis of 408 patients with the use of ten molecular markers. J Thorac Cardiovasc Surg 1999, 117:736-743.
6. Suzuki K, Nagai K, Yoshida J, Moriya E, Nishimura M, Takahashi K, Nishikawa Y: Prognostic factors in clinical stage I non-small cell lung cancer. Ann Thorac Surg 1999, 67:927-932.
7. Liu D, Huang C, Kameyama K, Hayashi E, Yamauchi A, Kobayashi S, Yokosime H: E-cadherin expression associated with differentiation and prognosis in patients with non-small cell lung cancer. Ann Thorac Surg 2001, 71:949-955.
8. D’Amico TA, Aloia TA, Moore MB, Herndon JE 2nd, Brooks KR, Lau CL, Harpole DH Jr: Molecular biologic staging of stage I lung cancer according to gender and histology. Ann Thorac Surg 2000, 69:882-886.
9. Shopland DR: Tobacco use and its contribution to early cancer mortality with a special emphasis on cigarette smoking. Environ Health Perspect 1995, 103(1):1-12.
10. Esposito V, Baldi A, De Luca A, Mazzarella G, Micheli P, Baldi F, Caputi M, Giordano A: Prognostic value of p53 in non small cell lung cancer: relationship with proliferating cell nuclear antigen and cigarette smoking. Human Path 1997, 28:233-237.
11. Esposito V, Baldi A, De Luca A, Groeger AM, Loda M, Giordano GG, Caputi M, Baldi F, Pagano M, Giordano A: Prognostic role of the cyclin-dependent kinase inhibitor p27 in non small lung cancer.. Cancer Res 1997, 57:3381-3385.
12. Caputi M, Esposito V, Baldi A, De Luca A, Dean C, Signoriello G, Baldi F, Giordano A: p21 expression in non-small cell lung cancer: relationship to survival. Anticancer Res 1998, 18:213-217.
13. Groeger AM, Caputi M, Esposito V, Baldi A, Rossielo R, Santini D, Mancini A, Kaiser HE, Baldi F: Expression of p21 in non-small cell lung cancer: relationship with PCNA. Anticancer Res 2000, 20:3301-3306.
14. Baldi A, Esposito V, De Luca A, Fu Y, Meoli I, Giordano GG, Caputi M, Baldi F, Giordano A: Differential expression of Rb2/p130 and p107 in normal human tissue and primary lung cancer. Clin Cancer Res 1997, 3:1691-1697.
15. Esposito V, Baldi A, Liuzzi G, Tonini G, Vincenzi B, Persichetti P, Santini M, Ambrogi V, Mineo TC, Montesarchio V, Wolner E, Baldi F, Groeger AM: Analysis of expression of angiopoietin-1 (Ang-1) and transforming growth factor-beta (TGF-beta) in resected non small cell lung cancer. Histopathology 2003, 43:901-905.

16. Groeger AM, Esposito V, De Luca A, Cassandro R, Tonini G, Ambrogi V, Baldi F, Goldfarb R, Mineo TC, Baldi A, Wolner E: Prognostic value of immunohistochemical expression of p53, BAX, BCL-2 and BCL-XL in resected non small cell lung cancer. Histopathology 2004, 45:54-63.

17. Esposito V, Baldi A, De Luca A, Tonini G, Vincenzi B, Santini D, Persichetti P, Mancini A, Citro G, Baldi F, Groeger AM, Caputi M: Cell cycle related proteins as prognostic parameters in radically resected non small cell lung cancer (NSCLC). J Clin Pathol 2005, 58:734-739.

18. Mineo TC, Ambrogi V, Baldi A, Rabitti C, Bollerio P, Vincenzi B, Tonini G: Prognostic impact of VEGF, CD31, CD34, and CD105 expression and tumor vessel invasion after radical surgery for IB-IIA non-small cell lung cancer. J Clin Pathol 2004, 57:591-597.

19. Esposito V, Campioni M, De Luca A, Spugnini EP, Baldi F, Cassandro R, Mancini A, Vincenzi B, Groeger A, Caputi M, Baldi A: Analyses of HtrA1 serine protease expression in human lung cancer. J Cancer Res Clin Oncol 2006, 132:345-3460.

20. Baldi A, Battista T, De Luca A, Santini D, Rossellio R, Baldi F, Natali PG, Lombardi D, Piccardo M, Felsani A, Paggi MG: Identification of genes down-regulated during melanoma progression: a cDNA array study. Exp Dermatol 2003, 12:213-218.

21. Vincenzi B, Schiavon G, Tonini G, Santini D, Persichetti P, Mancini A, Citro G, Baldi F, Groeger AM, Caputi M: Cell cycle related proteins as prognostic parameters in radically resected non small cell lung cancer (NSCLC). J Clin Pathol 2003, 2005, 87:5-9.

22. Maccharini P, Fontanini G, Hardin PJ, Squartini F, Angeletti CA: Relation of neovascularization to metastasis of non-small cell lung cancer. Cancer 1992, 40:145-146.

23. Yano T, Tanikawa S, Fujie T, Masutani M, Horie T: Vascular endothelial growth factor expression and neovascularisation in non-small cell lung cancer. Eur J Cancer 2000, 36:601-609.

24. Offersten BV, Pfeiffer P, Hamilton-Dutoit S, Overgaard J: Patterns of angiogenesis in non-small-cell lung carcinoma. Cancer 2001, 91:1500-1509.

25. Kuryayoshi M, Oue N, Yamamoto H, Kishida M, Inoue A, Asahara T, Kurayoshi M, Oue N, Yamamoto H, Kishida M, Inoue A, Asahara T, Kishida M, Inoue A, Asahara T: Semaphorin SEMA3B induces apoptosis in lung and breast cancer, whereas VEGF165 antagonizes this effect. Proc Natl Acad Sci USA 2004, 101:11432-11437.

26. McDoniels-Silvers AL, Nimri CF, Stoner GD, Lubet RA, You M: Differential gene expression in human lung adenocarcinomas and squamous cell carcinomas. Clin Cancer Res 2002, 8:1127-1138.

27. Shintani K, Matsunine A, Kusuzaki K, Morikawa J, Matsubara T, Wakahayashi T, Araki K, Satonaka H, Wakahayashi H, Iino T, Uchida A: Decorin suppresses lung metastases of murine osteosarcoma. Oncol Rep 2008, 19:1533-1539.

28. Ryk C, Kuma R, Thirumaran RK, Hou SM: Polymorphisms in the DNA repair genes XRCC1, APEX1, XRCC3 and NBS1, and the risk for lung cancer in never- and ever-smokers. Lung Cancer 2006, 54:285-292.

29. López-Cimà MF, González-Arriga P, García-Castro L, Pascual T, Marron MG, Puente X, Tardón A: Polymorphisms in XPC, XPD, XRCC1, and XRCC3 DNA repair genes and lung cancer risk in a population of northern Spain. BMC Cancer 2007, 7:162.

30. Weaver DA, Crawford EL, Warner KA, Elkhairi F, Khuder SA, Willey JC: ABCG5, ABCG1, XPA and XRCC1 transcript abundance levels correlate with cisplatin chemoresistance in non-small cell lung cancer cell lines. Mol Cancer 2005, 4:18.

31. Shintani K, Matsunine A, Kusuzaki K, Morikawa J, Matsubara T, Wakahayashi T, Araki K, Satonaka H, Wakahayashi H, Iino T, Uchida A: Decorin suppresses lung metastases of murine osteosarcoma. Oncol Rep 2008, 19:1533-1539.

32. Ryk C, Kuma R, Thirumaran RK, Hou SM: Polymorphisms in the DNA repair genes XRCC1, APEX1, XRCC3 and NBS1, and the risk for lung cancer in never- and ever-smokers. Lung Cancer 2006, 54:285-292.

33. López-Cimà MF, González-Arriga P, García-Castro L, Pascual T, Marron MG, Puente X, Tardón A: Polymorphisms in XPC, XPD, XRCC1, and XRCC3 DNA repair genes and lung cancer risk in a population of northern Spain. BMC Cancer 2007, 7:162.

34. Weaver DA, Crawford EL, Warner KA, Elkhairi F, Khuder SA, Willey JC: ABCG5, ABCG1, XPA and XRCC1 transcript abundance levels correlate with cisplatin chemoresistance in non-small cell lung cancer cell lines. Mol Cancer 2005, 4:18.

35. McDoniels-Silvers AL, Nimri CF, Stoner GD, Lubet RA, You M: Differential gene expression in human lung adenocarcinomas and squamous cell carcinomas. Clin Cancer Res 2002, 8:1127-1138.

36. Shintani K, Matsunine A, Kusuzaki K, Morikawa J, Matsubara T, Wakahayashi T, Araki K, Satonaka H, Wakahayashi H, Iino T, Uchida A: Decorin suppresses lung metastases of murine osteosarcoma. Oncol Rep 2008, 19:1533-1539.

37. Ryk C, Kuma R, Thirumaran RK, Hou SM: Polymorphisms in the DNA repair genes XRCC1, APEX1, XRCC3 and NBS1, and the risk for lung cancer in never- and ever-smokers. Lung Cancer 2006, 54:285-292.

38. López-Cimà MF, González-Arriga P, García-Castro L, Pascual T, Marron MG, Puente X, Tardón A: Polymorphisms in XPC, XPD, XRCC1, and XRCC3 DNA repair genes and lung cancer risk in a population of northern Spain. BMC Cancer 2007, 7:162.

39. Weaver DA, Crawford EL, Warner KA, Elkhairi F, Khuder SA, Willey JC: ABCG5, ABCG1, XPA and XRCC1 transcript abundance levels correlate with cisplatin chemoresistance in non-small cell lung cancer cell lines. Mol Cancer 2005, 4:18.

40. McDoniels-Silvers AL, Nimri CF, Stoner GD, Lubet RA, You M: Differential gene expression in human lung adenocarcinomas and squamous cell carcinomas. Clin Cancer Res 2002, 8:1127-1138.