Saudi Pharmaceutical Journal 25 (2017) 1151–1157

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Prediction of *Chlamydia pneumoniae* protein localization in host mitochondria and cytoplasm and possible involvements in lung cancer etiology: a computational approach

Aws Alshamsan [a,b,*,1], Shahanavaj Khan [a,*,1], Ahamad Imran [b], Ibrahim A. Aljuffali [a], Khalid Alsaleh [c]

[a] Nanomedicine Research Unit, Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[b] King Abdullah Institute for Nanotechnology, King Saud University, Riyadh, Saudi Arabia
[c] Oncology Center, King Saud Medical City, King Saud University, Riyadh, Saudi Arabia

ARTICLE INFO

Article history:
Received 15 December 2016
Accepted 30 May 2017
Available online 31 May 2017

Keywords:
Lung cancer
Chlamydia pneumoniae
Etiology
Systems biology
Protein targeting

ABSTRACT

Collecting evidence suggests that the intercellular infection of *Chlamydia pneumoniae* in lungs contributes to the etiology of lung cancer. Many proteins of *Chlamydia pneumoniae* outmanoeuvre the various system of the host. The infection may regulate various factors, which can influence the growth of lung cancer in affected persons. In this *in-silico* study, we predict potential targeting of *Chlamydia pneumoniae* proteins in mitochondrial and cytoplasmic comportments of host cell and their possible involvement in growth and development of lung cancer. Various cellular activities are controlled in mitochondria and cytoplasm, where the localization of *Chlamydia pneumoniae* proteins may alter the normal functioning of host cells. The rationale of this study is to find out and explain the connection between *Chlamydia pneumoniae* infection and lung cancer. A sum of 183 and 513 proteins were predicted to target in mitochondria and cytoplasm of host cell out of total 1112 proteins of *Chlamydia pneumoniae*. In particular, many targeted proteins may interfere with normal growth behaviour of host cells, thereby altering the decision of program cell death. Present article provides a potential connection of *Chlamydia pneumoniae* protein targeting and proposed that various targeted proteins may play crucial role in lung cancer etiology through diverse mechanisms.

1. Introduction

Lung cancer is one of the main reasons for cancer-related mortalities world-wide (Siegel et al., 2016). The association of *Chlamydia pneumonia (C. pneumonia)* infections with lung cancer etiology has been previously suggested (Byrne and Ojcius, 2004; Laurila et al., 1997). However, the process of carcinogenesis is not well understood yet. *C. pneumoniae* is a widespread intracellular respiratory bacterium that needs host cell for their existence and multiplication within host cells. It has evolved various effector proteins and toxins that interfere with cell death signalling pathways in host cells and the machinery of cell death executioner (Bohme and Rudel, 2009). Nucleus, mitochondria, and cytoplasm are critical components for normal host-cell functions (Bhavsar et al., 2007; Hess et al., 2003). Mitochondria, which are generally considered to originate from endosymbiotic event, plays an important role in numerous biochemical cascades that direct to programmed cell death (Gray et al., 1999). Therefore, investigating the mechanisms by which intracellular pathogens such as *C. pneumoniae* may alter apoptotic pathways is of special interest.

We have recently studied the nuclear targeting of *C. pneumoniae* proteins through computational approach during intracellular infection where they may play crucial role in progress and development of lung cancer (Khan et al., 2016a). Nevertheless, the fact that many *C. pneumoniae* proteins are targeted into mitochondria, cytoplasm, and other host intracellular components may alter many biochemical pathways in the host. Here we systematically predict several *C. pneumoniae* proteins that can localize into host

* Corresponding authors at: Nanomedicine Research Unit, Department of Pharmaceutics, College of Pharmacy, PO Box 2457, King Saud University, Riyadh 11451, Saudi Arabia.
E-mail address: aalshamsan@ksu.edu.sa (A. Alshamsan).
[1] These authors contributed equally to this work.
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2017.05.007
1319-0164/

mitochondria and cytoplasm where they can influence the development and progress of lung cancer.

2. Materials and methods

2.1. Protein database for the selection of C. pneumoniae strain

The Uniprot database was screened out for the selection of *C. pneumoniae* strain. Five strains *C. pneumoniae* are publicly accessible in Uniprot database with different number of proteins (Kalman et al., 1999; Myers et al., 2009; Read et al., 2000; Shirai et al., 2000). The *C. pneumoniae* pathogen is reported as obligate gram negative bacteria which may act as a potential factor of lung cancer (Yang et al., 2003; Zhan et al., 2011).

2.2. cNLS mapper for the analysis of NLS

The bioinformatics software cNLS mapper was utilized for the analysis of nuclear localization signal (NLS) in whole proteins of *C. pneumoniae* TW-183 strain (Kosugi et al., 2009a). The whole sequence of all proteins was utilized for the analysis of monopartite and bipartite NLS sequence cutoff value.

2.3. BaCelLo for the analysis of sub cellular protein targeting in different cell compartments

Protein targeting of *C. pneumoniae* TW-183 stain in different eukaryotic cell organelles were analysed by bioinformatics software Balanced Sub Cellular Localization predictor (BaCelLo). The BaCelLo software based on three well known datasets for eukaryotic kingdoms which includes plants, fungi, and animals (Pierleoni et al., 2006). The software analyses the five different classes of sub cellular localization such as mitochondrial, cytoplasmic, nuclear, chloroplast and secretory. The current study was performed with animal datasheet related tool.

3. Results

3.1. Protein database for the selection of C. pneumoniae strain

Whole proteins of *C. pneumoniae* (TW-183 strain) were analysed in present study. We selected *C. pneumoniae* TW-183 strain for our prediction study because of its contains the largest proteome, total of 1112 proteins, as compared to the other four *C. pneumoniae* strains J138, CWL029, AR39 and LPCoLN (Kalman et al., 1999; Myers et al., 2009; Read et al., 2000; Shirai et al., 2000). Scheme showed the summary of work plan for data analysis and possible outcome. Detailed description was presented in the experimental, result and discussion section (Fig. 1).

3.2. Prediction of mitochondrial targeting of C. pneumoniae proteins

The prediction results of BaCelLo have shown 183 proteins target to host cell mitochondria out of total 1112 proteins. It was found that raising bipartite NLS cutoff value of proteins was associated with high probability to targeting in mitochondria. On the contrary, an opposite pattern was noticed with monopartite NLS value of predicted proteins (Table 1). However, no correct connection was predicted with molecular weight and protein targeting to mitochondrial except in few cases where the proteins with minimum molecular weight (0–20 kDa) were mainly localized in host-cell mitochondria (Table 2). The results of pI values were not indicating any constant pattern for targeting of *C. pneumonia* proteins in host mitochondria (Table 3). Multi-modality of pI distribution is a common feature in different whole proteomes. Some researchers studied pI value corelate to the protein localization in different subcellular compartments (Wu et al., 2006). The outlines of proteins localization in host mitochondria illustrated in Fig. 2. Furthermore, as shown in (Table S1), we have provided details about 183 proteins predicted to target mitochondria during our study.

3.3. Prediction of cytoplasmic targeting of C. pneumoniae proteins

The prediction results of BaCelLo have shown 513 proteins targeted to host cell cytoplasm out of total 1112 proteins of whole *C. pneumoniae* proteome. Most of the proteins containing NLS cutoff value of 3–5 were targeted to the cytoplasm with respect to monopartite NLS and bipartite NLS cutoff values (Table 1). Also, most proteins with higher molecular weights were associated host-cell cytoplasmic localization with few exceptions (Table 2). On the other hand, it was observed that the increasing the pI value of the predicted proteins constantly decreased the localization in cytoplasm of host cell (Table 3). The localization patterns of *C. pneumoniae* proteins in host cytoplasm are illustrated in Fig. 3 with different parameters. Moreover, additional data provides the description regarding various *C. pneumoniae* proteins targeting to cytoplasm of host cell are listed in (Table S2).

4. Discussion

Various bacterial proteins modulate host cell survival leading to dynamical suppression of host cell death as a mean for bacterial persistence and multiplication (Fan et al., 1998; Fischer et al., 2001; Rajalingam et al., 2001; Ballestar and Esteller, 2005. *In silico* analysis of protein subcellular localization can significantly help to explain bacterial-protein potential functions. Although many advanced experimental high-throughput approaches have been developed to determine proteins localization, they are time consuming and cost ineffective (Huh et al., 2003; Khan et al., 2016b,2016c; Laurila and Vihinen, 2009; Mote and Reines, 1998). In the advancement of bioinformatics, fast and highly-accurate genome-scale computational predictors of subcellular protein targeting/localization offer a good complement for experimental practice (Garg et al., 2005; Reinhardt and Hubbard, 1998). cNLS Mapper predicts classical NLS functionality of proteins by analysing summation of the functional role of each amino acid in the query protein as per the activity-based profiles, which are achieved from the systematic residue-replacement analyses in *Saccharomyces cerevisiae*. Although analysis of NLS in a particular sequence of protein is essential for analysing its nuclear localization with the highest cNLS score, the cNLS Mapper is a predictor to analyse NLS activity rather than NLS sequence as it consequently predicts cytoplasmic localization of proteins (Hahn et al., 2008; Kosugi et al., 2009a, 2009b).

In contrast, BaCelLo is another predictor that utilizes a decision tree which was developed on diverse SVMs for the prediction of nuclear, mitochondrial, chloroplast, and cytoplasmic targeting of particular proteins (Pierleoni et al., 2006). Also, BaCelLo predicts complete protein sequence along with its N and C termini and analyse the results on the basis of information gained by amino acid residue sequence and evolutionary alignment. To impede different mitochondrial functions, bacterial proteins should attach to- and enter the mitochondria during the course of infection (Cossart and Sansonetti, 2004; Kozjak-Pavlovic et al., 2008). In addition to several anomalous mitochondrial functions, cancer cells have impaired oxidative phosphorylation as a result of the high-rate modified glycolysis with elevated fermentation of lactic acid, which permits cancer cells to retain biosynthetic fluxes at the time of fast proliferation (DeBerardinis et al., 2008; Rudel et al., 2010;

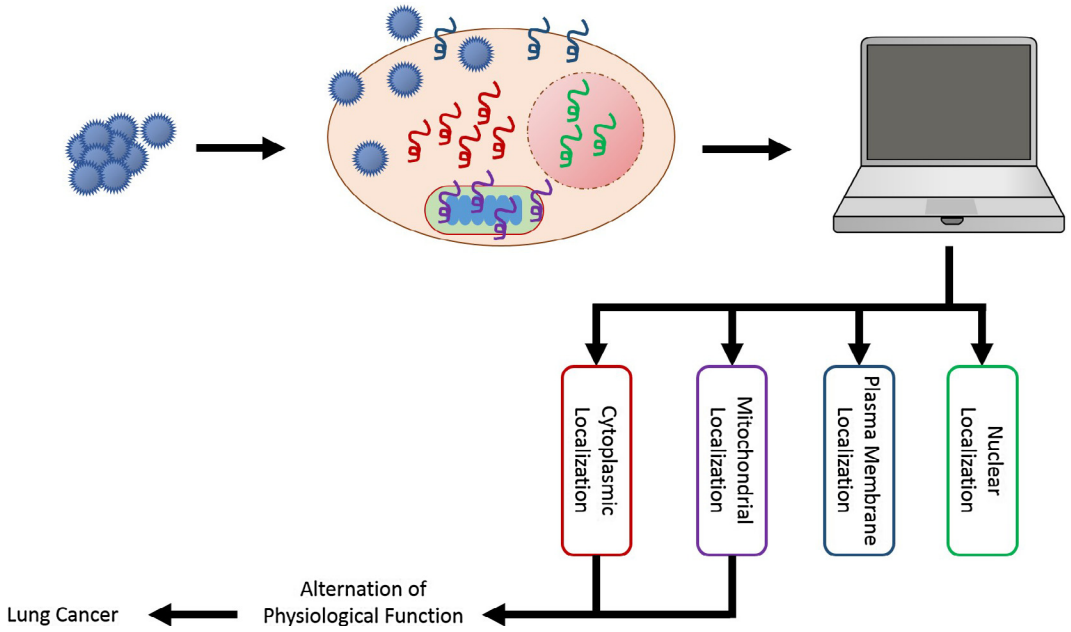

Fig. 1. Scheme illustrated the summary of work plan in current study during data prediction and analysis.

Table 1
Computational prediction of *Chlamydia pneumoniae* proteins targeting to mitochondria and cytoplasm of host cell and their relation with total proteins with similar NLS.

NLS	NLS Cutoff values	Number of proteins targeting mitochondria	Total number of proteins in this range	Percentage	NLS Cutoff values	Number of proteins targeting cytoplasm	Total number of proteins in this range	Percentage
Monopartite NLS	0–3.0	151	981	15.39	0–3.0	465	981	47.40
	3.0–5.0	13	64	20.31	3.0–5.0	22	64	34.27
	5.0–8.0	16	50	32	5.0–8.0	17	50	34
	>8.0	3	17	17.64	>8.0	09	17	52.94
Bipartite NLS	0–3.0	24	274	8.75	0–3.0	108	274	39.41
	3.0–5.0	109	616	17.69	3.0–5.0	305	616	49.51
	5.0–8.0	48	209	22.96	5.0–8.0	93	209	44.49
	>8.0	2	13	15.38	>8.0	07	13	53.84

Table 2
Computational prediction of *Chlamydia pneumoniae* proteins targeting to mitochondria and cytoplasm of host cell and their relation with total proteins with similar molecular weight.

Molecular weight	Number of proteins targeting to mitochondria	Total number of proteins	Percentage	Molecular weight	Number of proteins targeting to cytoplasm	Total number of proteins	Percentage
0–20 kD	65	326	19.93	0–20 kD	102	326	31.28
20–40 kD	67	384	17.44	20–40 kD	191	384	49.73
40–60 kD	36	235	15.31	40–60 kD	126	235	53.61
60–80 kD	10	87	11.49	60–80 kD	56	87	64.36
>80 kD	5	80	6.25	>80 kD	38	80	47.50

Table 3
Computational prediction of *Chlamydia pneumoniae* proteins targeting to mitochondria and cytoplasm of host cell, and their relation with total proteins with similar pI value.

Range of pI value	Number of proteins targeting to mitochondria	Total number of proteins	Percentage	Range of pI value	Number of proteins targeting to cytoplasm	Total number of proteins	Percentage
3.0–5.0	4	106	3.77	3.0–5.0	58	106	4.71
5.0–6.0	22	297	7.40	5.0–6.0	187	297	62.96
6.0–7.0	20	175	11.42	6.0–7.0	96	175	54.85
7.0–8.0	10	79	12.65	7.0–8.0	38	79	48.10
8.0–9.0	24	150	16	8.0–9.0	59	150	29.33
9.0–10	61	215	28.37	9.0–10	52	215	24.18
10.0–11.0	30	70	42.85	10.0–11.0	17	70	24.28
11.0–13.0	12	20	60	11.0–13.0	6	20	30

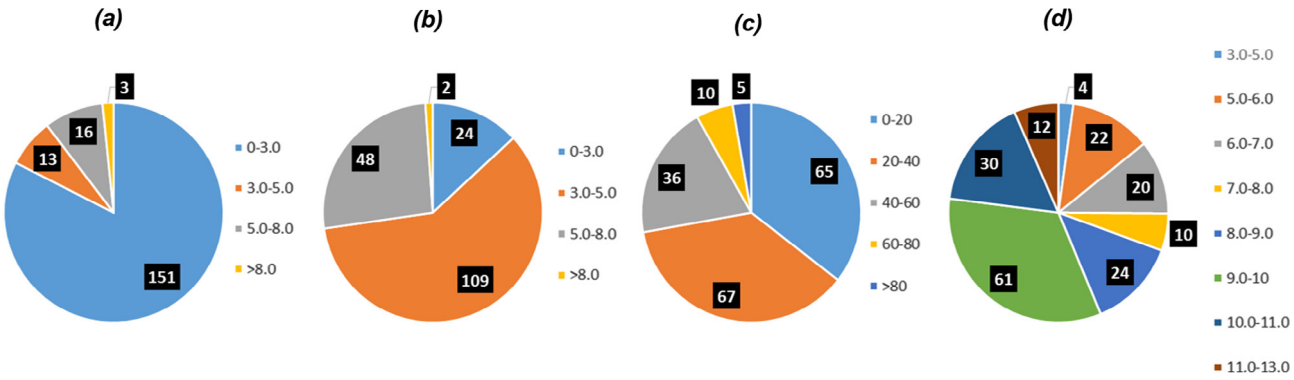

Fig. 2. *C. pneumoniae* targeting proteins in host mictochondria as per different range of monopartite NLS cut-off value (a), bipartite NLS cut-off value (b), molecular weight (c), and pI value (d).

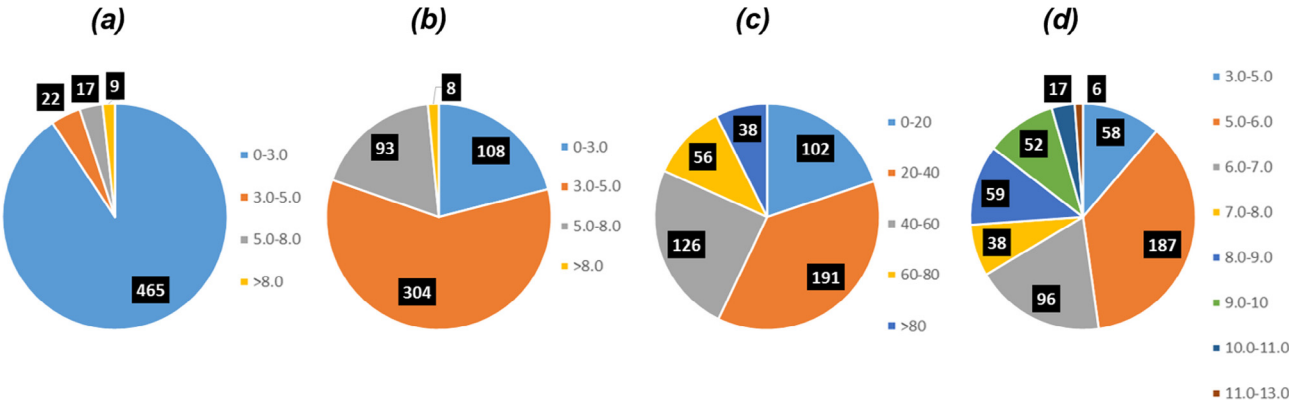

Fig. 3. *C. pneumoniae* targeting proteins in host cytoplasm as per different range of monopartite NLS cut-off value (a), bipartite NLS cut-off value (b), molecular weight (c), and pI value (d).

Wallace, 2012). Nevertheless, cancer cells showed antagonistic properties towards mitochondria-mediated apoptotic pathways (Sabharwal and Schumacker, 2014; Wallace, 2012). In the current *in silico* analysis, we predicted a sum of 183 proteins out of total 1112 proteins of *C. pneumoniae* targets to host-cell mitochondria, which may alter mitochondrial functions to the favour of oncogenesis.

We analysed bacterial DNA-binding protein HU due to its potential of influencing mitochondrial protein expression. As they share properties with histone proteins, the potential involvement of DNA-binding proteins in the growth and progression of cancer have been revealed in various studies. Methyl CpG binding protein has the potential to interfere with methylation of target DNA and involved as a crucial factor for the progression of cancer (Ballestar and Esteller, 2005; Ghoshal and Bai, 2007). Chromodomain helicase DNA binding protein 1-like (CHD1L) plays a critical role in pathogenesis of hepatocellular carcinoma (Clemencon et al., 2013; Li et al., 2013). Similarly, Chromodomain helicase DNA binding protein 5 (CHD5) is an effective tumor repressor that potentially engaged in the growth of lung cancer due to their function inactivation by epigenetic mechanism (Silvera et al., 2010; Zhao et al., 2012). In general, bacterial DNA-binding proteins may interfere with ability of host DNA-binding proteins to interact with host DNA. It has been observed that over expression of inhibitor of DNA-binding protein (ID1) and vascular endothelial growth factor (VEGF) is associated with the growth and progression of various type of cancer (Georgiadou et al., 2014; Kubelac et al., 2014; Grzmil and Hemmings, 2012). Moreover, our findings show that many gene expression regulators can target host mitochondria. These proteins include, but are not limited to, transcription initiation factor sigma D protein (Q9Z8I1), transcription repressor protein HrcA (Q9Z850), endonuclease IV (Q9Z7H3), tRNA-ribosyltransferase (Q9Z8W5), DNA-directed DNA polymerase III chain (Q7VQ53), Methionyl-tRNA formyltransferase (Q9Z7Q5), DNA repair protein RecO (Q9Z7W5), Ribonuclease H-III (Q9Z6J1), ribosome-binding factor A (Q9Z8M0), RNA methyltransferase (Q9Z7N0), endonuclease III (Q9Z769), translation initiation factor IF-3 (Q9Z6R9).

Mitochondria have their own genetic material and protein-synthesizing machinery, which more closely resembles bacteria. It has been examined in a study that bacterial RNA polymerase have the capability to act upon host DNA sequences due to conserved nature of RNA polymerase II and bacterial RNA polymerases (Kechavarzi and Janga, 2014; Mote and Reines, 1998). Another study illustrated conserved mechanisms for substrate binding and nucleotide integration with bacteriophage RNA polymerase (Kim and Lee, 2009; Schwinghammer et al., 2013). Similarly, the possible function of transcription termination proteins of *C. pneumoniae* on human transcription termination genes should be examined prior to any conclusion. Although enormous difference is observed among the mechanisms of transcription termination in human and bacterial cells, it is found plausible that many RNA polymerase II transcribed genes of eukaryotes use attenuation and antitermination processes as same as bacteria (Boland et al., 2013). Therefore, we predicted the localization of transcription termination associated factor IF-3 and DNA-directed DNA polymerase III chain in host mitochondria.

Moreover, the DNA-binding proteins we predicted may also interfere with other existing mitochondrial gene regulation proteins in host cells through binding with particular sequences as they may alter the expression level of various genes. Many reports revealed that alteration in the replication and/or expression of

mitochondria is connected with progression of many type of cancer including lung cancer (Giang et al., 2013; Shapovalov et al., 2011; Wang et al., 2015; Verstraeten et al., 2011). Mutations in the mitochondrial DNA (mtDNA) have long been supposed to play a crucial role in cancer etiology including lung cancer (Brandon et al., 2006). Earlier reports showed that approximately 43% of lung cancers had mtDNA mutations (Fliss et al., 2000). The mtDNA is more common for mutations because of reduced fidelity of DNA polymerase of mitochondria, increased level of generation of endogenous ROS, and lack of effective repair mechanisms for mtDNA (Maximo et al., 2009). Although our results revealed the potential of various *C. pneumoniae* proteins to enter host mitochondria and create mutation in host mtDNA and subsequently alter gene expression, the limited information of *in silico* studies required accurate experimental support prior to conclusion. Furthermore results with other proteins are illustrated in Table S1.

The metabolism of cancer cell characterized by elevated rate of glycolysis in presence of oxygen (Koppenol et al., 2011). This event, noticed by O. Warburg in 1924 and believed as the result of a "damaged" metabolism (Warburg, 1956). Our results indicated that *C. pneumoniae* enzymes glyceraldehyde 3 phosphate dehydrogenase (GAPDH, Accession no. Q9Z7T0) and acetyltransferase (Accession no. Q9Z7X8) localize to host mitochondria. Same enzymes from diverse species may exert enormous effect on particular substrates. This phenomenon is highly liable with an enzyme or protein from two distant evolutionary progenitors such as *C. pneumoniae* and human. Deregulation in glycolysis due to dysfunction of GAPDH has been associated with declined apoptotic signals in several types of cancer including lung cancer (Barbini et al., 2007; Krasnov et al., 2013; Tokunaga et al., 1987). Similarly, dysfunction of acetyltransferase was associated with lung cancer etiology (Zhao et al., 2013). Other *C. pneumoniae* enzymes such as endonuclease III and IV are also potentially target host cell mitochondria. Endonuclease III and IV have homology with human NTH1 and APE1 respectively (Hilbert et al., 1997; Marenstein et al., 2004). Up regulation in the expression of mitochondrial NTH1 and APE-1 is a characteristic for many type of cancers included lung cancer (Karahalil et al., 2010; Yoo et al., 2008). Therefore, experimental evidence should be evaluated on the basis of such prediction.

Similarly, cytoplasmic localization of *C. pneumoniae* proteins host cell may exert several effects which may lead to oncogenesis. Our study predicted a variety of *C. pneumoniae* proteins localized to cytoplasm of host cell as indicated in (Table S2). Among which, some proteins may alter the normal functioning of mRNA, tRNA, ribosome, translation, nucleotide synthesis and nucleotide degradation. We explored the cytoplasmic localization of some *C. pneumoniae* enzymes including aspartyl-tRNA synthetase (Q9Z7P2) and uridylate kinases (Q9Z7K7). The enzyme aspartyl-tRNA synthetase is a part of aminoacyl-tRNA synthetases (ARSs) multi-enzyme complex, which is involved in protein synthesis. It is reported that twenty types of ARSs connect each amino acid to their related tRNAs. Particular ARSs are linked with various diseases condition including autoimmune diseases, neuronal diseases, and cancer (Kim et al., 2014, 2011). Moreover, altered level of expression of uridylate kinases (Q9Z7K7), which is involved in the pryimeden nucleoside biosynthesis, was associated with multiple types of cancer (Humeniuk et al., 2009; Xu et al., 2008).

Several studies confirmed the involvement of various types of proteases in cancer progression through influencing angiogenesis cell adhesion, migration and proliferation (de Aberasturi and Calvo, 2015; Koblinski et al., 2000). We analysed ATP-dependent Clp protease (Q9Z760), methionine aminopeptidase (Q9Z6Q0), ATP-dependent zinc metalloprotease (Q9Z6R1), and ATP-dependent protease La (Q9Z9F4). These enzymes are involved in peptide and abnormal protein degradation. In addition to proteases, methyltransferases, enzymes that catalyse site-specific methylation, are associated with various types of oncogenesis (Copeland, 2013). We investigated host-cell cytoplasmic localization of *C. pneumoniae* protoporphyrinogen oxidase (Q7VQ81), DNA methyltransferase (Q9Z7V8), tRNA (guanine-N(7)-)-methyl transferase (Q9Z6S3), and SAM dependent methyltransferase (Q9Z821) cytidine/uridine-2′-O-)-methyltransferase (Q9Z7P4). The inactivation of protoporphyrinogen oxidase is associated with liver cancer in acute porphyries (Schneider-Yin et al., 2015). Similarly, DNA methyltransferases performed the process of methylation and a potential targets for cancer therapy (Ghoshal and Bai, 2007). Moreover, ADP/ATP carrier protein 2 (Q9Z7U0) predicted in cytoplasmic localization, which act as a decisive component in apoptosis and demonstrated recently to have an important role in cancer (Clemencon et al., 2013). In addition, nucleoside diphosphate kinase (NDK) (Q9Z7T5), is also analysed to localize in the cytoplasm of host cell. NDK is involved in cell growth differentiation, proliferation, and tumor metastasis. Furthermore, it is also important for the metabolism and synthesis of nucleotide. The presence of enzyme NDK in host cytoplasm has been implicated in cancer etiology (Kimura et al., 2000). Nevertheless the exact role of NDK in cancer needs further appraisal. Thus, it might be possible that the expected future of a cell is dictated by various factors of host and microbiota, including bacteria, in order to transform a normal cell into cancerous cell.

Translation is a very important process to control the normal expression of several proteins. The possible involvement of translation-associated proteins in growth and advancement of cancer was examined and confirmed in numerous studies (Bhat et al., 2015; Ruggero, 2013). In our study, various ribosomal proteins of *C. pneumoniae* were predicted to localize in cytoplasm of host cell, which also acts as RNA-binding proteins. For instant, 30S ribosomal protein S1 (Q9Z8M3), S6 (Q9Z6V5), S5 (Q9Z7S3), and S20 (Q9Z7F2) are needed to express the mRNA with Shine–Dalgarno sequence. Similarly, various 50S ribosomal proteins are predicted in our study including L10 (Q9Z9A2), L9 (Q9Z6V3), L4 (Q9Z7Q8), L5 (Q9Z7R9), L29 (Q9Z7R5), L27 (Q9Z807), L33 (Q9Z8T4), L34 (Q9Z6X1) and L35 (Q9Z6R8), L36 (Q9Z6X0), which potentially involved in various steps of translation and ribosome biogenesis in *C. pneumoniae*. Control of translation is a critical component for the progress and advancement of cancer (Bhat et al., 2015; Silvera et al., 2010). It was reported that hyper-activated signalling pathways potentially manipulate the translation, which may be permitting of uncontrolled growth of cells (Grzmil and Hemmings, 2012). The cytoplasmic localization of *C. pneumoniae* enzymes and protein, which were observed in our study, showed the capability to interact with various regulatory elements and RNA of the host. Several RNA-binding proteins were shown to play a significant role in cancer development through regulation of gene expression at the post-transcriptional level (Grzmil and Hemmings, 2012; Kechavarzi and Janga, 2014). Many recent studies reported the involvement of various RNA-binding enzymes in cancer progression (van Kouwenhove et al., 2011; Wurth, 2012).

A category of enzymes are ribonucleases that cleave the phosphodiester bond of RNA and directing to many types of cellular consequences in host cell. It is evident that mammalian ribonucleases are involved in both prevention and development of various types of cancer (Kim and Lee, 2009). Nevertheless, the possible involvement of bacterial ribonucleases during their infection in oncogenesis is not much identified and requires precise biological corroborations. In our study, various ribonucleases such as ribonuclease III (Q9Z9C7), ribonuclease Z (Q9Z9F6), ribonuclease G (Q9Z6U9), and ribonuclease HII (Q9Z962) were analysed to localize in cytoplasm of host cell. Ribonuclease III (Q9Z9C7) is involved in processing mRNA, rRNA, and tRNA, whereas ribonuclease Z (Q9Z9F6) is involved in 3′-tRNA processing activity. In addition,

ribonuclease HII (Q9Z962) and ribonuclease G (Q9Z6U9) are showing RNA-DNA hybrid ribonuclease activity in *C. pneumoniae*. Hence, ribonuclease-activated prodrugs emerged as a new line of investigation (Ellis et al., 2012). GTPase HflX (Q7VQ01) is another RNA-binding protein predicted in our study. GTPase have the ability to hydrolyse the GTP and potentially involved in infection-induced oncogenesis (Verstraeten et al., 2011). Nevertheless, further more suitable experimental validation for *C. pneumoniae* cytoplasmic localization in human and their potential role in lung cancer etiology.

Altered level of expression of heat shock proteins (HSP) in different types of cancer have been investigated in several studies (Calderwood and Gong, 2012; Calderwood et al., 2006). Here, we predicted the cytoplasmic localization of various conserved HSP including HSP-70 (P27542), 60 kDa chaperonin (GroEL protein) (P31681), and Protein GrpE (HSP-70 cofactor) (Q9Z849), which may be engaged in accurate protein folding during adverse conditions in *C. pneumoniae*. For instant, Hsp70 shares about 50% sequence identity between *C. pneumoniae* and human whereas many domains showed 96% identities (Schlesinger, 1990; Zugel and Kaufmann, 1999). It has been revealed in several reports that bacterial HSPs helps in protection of epithelial cells from apoptosis and may enhance the process of cell proliferation (Zhang et al., 2004, 2001). The involvement of bacterial HSPs in proliferation of cell and cytoplasmic targeting of *C. pneumoniae* HSPs in lung cells during infection enhance the possible risk of lung cancer. This potential association between *C. pneumoniae* infection and risk of lung cancer gained credibility in the past many years but no consensus has been reached.

5. Conclusion

Taken together, our current prediction results show that *C. pneumoniae* protein targeting host-cell mitochondria and cytoplasm may act as a potential factor for growth and development of lung cancer. We hypothesize that alteration in normal functioning of cells may induced the process of carcinogenesis due to host-pathogen protein-protein interaction through dysregulation in various pathways of mitochondria and cytoplasm with different strategies. Although the present research suggests explanations of the role of *C. pneumoniae* in the progress and advancement of lung cancer, experimental research are urgently needed to confirm and validate the *in-silico* results. These findings should open new avenues for clinical microbiologists and oncologists when dealing with *C. pneumoniae* infections.

Competing interests statement

The authors state no any competing interest related to present work.

Acknowledgement

This project was funded by the Research Groups Program (Research Group number RG-1436-027), Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsps.2017.05.007.

References

Ballestar, E., Esteller, M., 2005. Methyl-CpG-binding proteins in cancer: blaming the DNA methylation messenger. Biochem. Cell Biol. 83, 374–384.

Barbini, L., Rodriguez, J., Dominguez, F., Vega, F., 2007. Glyceraldehyde-3-phosphate dehydrogenase exerts different biologic activities in apoptotic and proliferating hepatocytes according to its subcellular localization. Mol. Cell. Biochem. 300, 19–28.

Bhat, M., Robichaud, N., Hulea, L., Sonenberg, N., Pelletier, J., Topisirovic, I., 2015. Targeting the translation machinery in cancer. Nat. Rev. Drug Discov. 14, 261–278.

Bhavsar, A.P., Guttman, J.A., Finlay, B.B., 2007. Manipulation of host-cell pathways by bacterial pathogens. Nature 449, 827–834.

Bohme, L., Rudel, T., 2009. Host cell death machinery as a target for bacterial pathogens. Microbes Infect. 11, 1063–1070.

Boland, M.L., Chourasia, A.H., Macleod, K.F., 2013. Mitochondrial dysfunction in cancer. Front. Oncol 3, 292.

Brandon, M., Baldi, P., Wallace, D.C., 2006. Mitochondrial mutations in cancer. Oncogene 25, 4647–4662.

Byrne, G.I., Ojcius, D.M., 2004. Chlamydia and apoptosis: life and death decisions of an intracellular pathogen. Nat. Rev. Microbiol. 2, 802–808.

Calderwood, S.K., Gong, J., 2012. Molecular chaperones in mammary cancer growth and breast tumor therapy. J. Cell. Biochem. 113, 1096–1103.

Calderwood, S.K., Khaleque, M.A., Sawyer, D.B., Ciocca, D.R., 2006. Heat shock proteins in cancer: chaperones of tumorigenesis. Trends Biochem. Sci. 31, 164–172.

Clemencon, B., Babot, M., Trezeguet, V., 2013. The mitochondrial ADP/ATP carrier (SLC25 family): pathological implications of its dysfunction. Mol. Aspects Med. 34, 485–493.

Copeland, R.A., 2013. Molecular pathways: protein methyltransferases in cancer. Clin. Cancer Res. 19, 6344–6350.

Cossart, P., Sansonetti, P.J., 2004. Bacterial invasion: the paradigms of enteroinvasive pathogens. Science 304, 242–248.

de Aberasturi, A.L., Calvo, A., 2015. TMPRSS4: an emerging potential therapeutic target in cancer. Br. J. Cancer 112, 4–8.

DeBerardinis, R.J., Lum, J.J., Hatzivassiliou, G., Thompson, C.B., 2008. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab. 7, 11–20.

Ellis, G.A., McGrath, N.A., Palte, M.J., Raines, R.T., 2012. Ribonuclease-activated cancer prodrug. ACS Med. Chem. Lett. 3, 268–272.

Fan, T., Lu, H., Hu, H., Shi, L., McClarty, G.A., Nance, D.M., et al., 1998. Inhibition of apoptosis in chlamydia-infected cells: blockade of mitochondrial cytochrome c release and caspase activation. J. Exp. Med. 187, 487–496.

Fischer, S.F., Schwarz, C., Vier, J., Hacker, G., 2001. Characterization of antiapoptotic activities of Chlamydia pneumoniae in human cells. Infect. Immun. 69, 7121–7129.

Fliss, M.S., Usadel, H., Caballero, O.L., Wu, L., Buta, M.R., Eleff, S.M., et al., 2000. Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. Science 287, 2017–2019.

Garg, A., Bhasin, M., Raghava, G.P., 2005. Support vector machine-based method for subcellular localization of human proteins using amino acid compositions, their order, and similarity search. J. Biol. Chem. 280, 14427–14432.

Georgiadou, D., Sergentanis, T.N., Sakellariou, S., Filippakis, G.M., Zagouri, F., Vlachodimitropoulos, D., et al., 2014. VEGF and Id-1 in pancreatic adenocarcinoma: prognostic significance and impact on angiogenesis. Eur. J. Surg. Oncol. 40, 1331–1337.

Ghoshal, K., Bai, S., 2007. DNA methyltransferases as targets for cancer therapy. Drugs Today (Barc) 43, 395–422.

Giang, A.H., Raymond, T., Brookes, P., de Mesy Bentley, K., Schwarz, E., O'Keefe, R., et al., 2013. Mitochondrial dysfunction and permeability transition in osteosarcoma cells showing the Warburg effect. J. Biol. Chem. 288, 33303–33311.

Gray, M.W., Burger, G., Lang, B.F., 1999. Mitochondrial evolution. Science 283, 1476–1481.

Grzmil, M., Hemmings, B.A., 2012. Translation regulation as a therapeutic target in cancer. Can. Res. 72, 3891–3900.

Hahn, S., Maurer, P., Caesar, S., Schlenstedt, G., 2008. Classical NLS proteins from Saccharomyces cerevisiae. J. Mol. Biol. 379, 678–694.

Hess, S., Peters, J., Bartling, G., Rheinheimer, C., Hegde, P., Magid-Slav, M., et al., 2003. More than just innate immunity: comparative analysis of Chlamydophila pneumoniae and Chlamydia trachomatis effects on host-cell gene regulation. Cell. Microbiol. 5, 785–795.

Hilbert, T.P., Chaung, W., Boorstein, R.J., Cunningham, R.P., Teebor, G.W., 1997. Cloning and expression of the cDNA encoding the human homologue of the DNA repair enzyme, Escherichia coli endonuclease III. J. Biol. Chem. 272, 6733–6740.

Huh, W.K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S., et al., 2003. Global analysis of protein localization in budding yeast. Nature 425, 686–691.

Humeniuk, R., Menon, L.G., Mishra, P.J., Gorlick, R., Sowers, R., Rode, W., et al., 2009. Decreased levels of UMP kinase as a mechanism of fluoropyrimidine resistance. Mol. Cancer Ther. 8, 1037–1044.

Kalman, S., Mitchell, W., Marathe, R., Lammel, C., Fan, J., Hyman, R.W., et al., 1999. Comparative genomes of Chlamydia pneumoniae and C. trachomatis. Nat. Genet. 21, 385–389.

Karahalil, B., Bohr, V.A., De Souza-Pinto, N.C., 2010. Base excision repair activities differ in human lung cancer cells and corresponding normal controls. Anticancer Res. 30, 4963–4971.

Kechavarzi, B., Janga, S.C., 2014. Dissecting the expression landscape of RNA-binding proteins in human cancers. Genome Biol. 15, R14.

Khan, S., Imran, A., Khan, A.A., Abul Kalam, M., Alshamsan, A., 2016a. Systems biology approaches for the prediction of possible role of Chlamydia pneumoniae proteins in the etiology of lung cancer. PLoS ONE 11, e0148530.
Khan, S., Zakariah, M., Palaniappan, S., 2016b. Computational prediction of Mycoplasma hominis proteins targeting in nucleus of host cell and their implication in prostate cancer etiology. Tumour Biol. 37, 10805–10813.
Khan, S., Zakariah, M., Rolfo, C., Robrecht, L., Palaniappan, S., 2016c. Prediction of mycoplasma hominis proteins targeting in mitochondria and cytoplasm of host cells and their implication in prostate cancer etiology. Oncotarget.
Kim, D., Kwon, N.H., Kim, S., 2014. Association of aminoacyl-tRNA synthetases with cancer. Top. Curr. Chem. 344, 207–245.
Kim, S., You, S., Hwang, D., 2011. Aminoacyl-tRNA synthetases and tumorigenesis: more than housekeeping. Nat. Rev. Cancer 11, 708–718.
Kim, W.C., Lee, C.H., 2009. The role of mammalian ribonucleases (RNases) in cancer. Biochem. Biophys. Acta. 1796, 99–113.
Kimura, N., Shimada, N., Fukuda, M., Ishijima, Y., Miyazaki, H., Ishii, A., et al., 2000. Regulation of cellular functions by nucleoside diphosphate kinases in mammals. J. Bioenerg. Biomembr. 32, 309–315.
Koblinski, J.E., Ahram, M., Sloane, B.F., 2000. Unraveling the role of proteases in cancer. Clin. Chim. Acta 291, 113–135.
Koppenol, W.H., Bounds, P.L., Dang, C.V., 2011. Otto Warburg's contributions to current concepts of cancer metabolism. Nat. Rev. Cancer 11, 325–337.
Kosugi, S., Hasebe, M., Matsumura, N., Takashima, H., Miyamoto-Sato, E., Tomita, M., et al., 2009a. Six classes of nuclear localization signals specific to different binding grooves of importin alpha. J. Biol. Chem. 284, 478–485.
Kosugi, S., Hasebe, M., Tomita, M., Yanagawa, H., 2009b. Systematic identification of cell cycle-dependent yeast nucleocytoplasmic shuttling proteins by prediction of composite motifs. Proc. Natl. Acad. Sci. USA 106, 10171–10176.
Kozjak-Pavlovic, V., Ross, K., Rudel, T., 2008. Import of bacterial pathogenicity factors into mitochondria. Curr. Opin. Microbiol. 11, 9–14.
Krasnov, G.S., Dmitriev, A.A., Snezhkina, A.V., Kudryavtseva, A.V., 2013. Deregulation of glycolysis in cancer: glyceraldehyde-3-phosphate dehydrogenase as a therapeutic target. Expert Opin. Ther. Targets 17, 681–693.
Kubelac, M.P., Fetica, B., Vlad, I.C., Fulop, A., Popa, A., Achimas-Cadariu, P., 2014. The role of inhibitor of DNA-binding 1 (ID-1) protein and angiogenesis in serous ovarian cancer. Anticancer Res. 34, 413–416.
Laurila, A.L., Anttila, T., Laara, E., Bloigu, A., Virtamo, J., Albanes, D., et al., 1997. Serological evidence of an association between Chlamydia pneumoniae infection and lung cancer. Int. J. Cancer 74, 31–34.
Laurila, K., Vihinen, M., 2009. Prediction of disease-related mutations affecting protein localization. BMC Genomics 10, 122.
Li, Y., Chen, L., Chan, T.H., Liu, M., Kong, K.L., Qiu, J.L., et al., 2013. SPOCK1 is regulated by CHD1L and blocks apoptosis and promotes HCC cell invasiveness and metastasis in mice. Gastroenterology 144 (179–191), e4.
Marenstein, D.R., Wilson 3rd, D.M., Teebor, G.W., 2004. Human AP endonuclease (APE1) demonstrates endonucleolytic activity against AP sites in single-stranded DNA. DNA Repair (Amst) 3, 527–533.
Maximo, V., Lima, J., Soares, P., Sobrinho-Simoes, M., 2009. Mitochondria and cancer. Virchows Arch. 454, 481–495.
Mote Jr., J., Reines, D., 1998. Recognition of a human arrest site is conserved between RNA polymerase II and prokaryotic RNA polymerases. J. Biol. Chem. 273, 16843–16852.
Myers, G.S., Mathews, S.A., Eppinger, M., Mitchell, C., O'Brien, K.K., White, O.R., et al., 2009. Evidence that human Chlamydia pneumoniae was zoonotically acquired. J. Bacteriol. 191, 7225–7233.
Pierleoni, A., Martelli, P.L., Fariselli, P., Casadio, R., 2006. BaCelLo: a balanced subcellular localization predictor. Bioinformatics 22, e408–16.
Rajalingam, K., Al-Younes, H., Muller, A., Meyer, T.F., Szczepek, A.J., Rudel, T., 2001. Epithelial cells infected with Chlamydophila pneumoniae (Chlamydia pneumoniae) are resistant to apoptosis. Infect. Immun. 69, 7880–7888.
Read, T.D., Brunham, R.C., Shen, C., Gill, S.R., Heidelberg, J.F., White, O., et al., 2000. Genome sequences of Chlamydia trachomatis MoPn and Chlamydia pneumoniae AR39. Nucleic Acids Res. 28, 1397–1406.
Reinhardt, A., Hubbard, T., 1998. Using neural networks for prediction of the subcellular location of proteins. Nucleic Acids Res. 26, 2230–2236.
Rudel, T., Kepp, O., Kozjak-Pavlovic, V., 2010. Interactions between bacterial pathogens and mitochondrial cell death pathways. Nat. Rev. Microbiol. 8, 693–705.
Ruggero, D., 2013. Translational control in cancer etiology. Cold Spring Harb Perspect Biol 5.
Sabharwal, S.S., Schumacker, P.T., 2014. Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles' heel? Nat. Rev. Cancer 14, 709–721.
Schlesinger, M.J., 1990. Heat shock proteins. J. Biol. Chem. 265, 12111–12114.
Schneider-Yin, X., van Tuyll van Serooskerken, A.M., Siegesmund, M., Went, P., Barman-Aksozen, J., Bladergroen, R.S., et al., 2015. Biallelic inactivation of protoporphyrinogen oxidase and hydroxymethylbilane synthase is associated with liver cancer in acute porphyrias. J. Hepatol. 62, 734–738.
Schwinghammer, K., Cheung, A.C., Morozov, Y.I., Agaronyan, K., Temiakov, D., Cramer, P., 2013. Structure of human mitochondrial RNA polymerase elongation complex. Nat. Struct. Mol. Biol. 20, 1298–1303.
Shapovalov, Y., Hoffman, D., Zuch, D., de Mesy Bentley, K.L., Eliseev, R.A., 2011. Mitochondrial dysfunction in cancer cells due to aberrant mitochondrial replication. J. Biol. Chem. 286, 22331–22338.
Shirai, M., Hirakawa, H., Kimoto, M., Tabuchi, M., Kishi, F., Ouchi, K., et al., 2000. Comparison of whole genome sequences of Chlamydia pneumoniae J138 from Japan and CWL029 from USA. Nucleic Acids Res. 28, 2311–2314.
Siegel, R.L., Miller, K.D., Jemal, A., 2016. Cancer statistics, 2016. CA Cancer J. Clin. 66, 7–30.
Silvera, D., Formenti, S.C., Schneider, R.J., 2010. Translational control in cancer. Nat. Rev. Cancer 10, 254–266.
Tokunaga, K., Nakamura, Y., Sakata, K., Fujimori, K., Ohkubo, M., Sawada, K., et al., 1987. Enhanced expression of a glyceraldehyde-3-phosphate dehydrogenase gene in human lung cancers. Can. Res. 47, 5616–5619.
van Kouwenhove, M., Kedde, M., Agami, R., 2011. MicroRNA regulation by RNA-binding proteins and its implications for cancer. Nat. Rev. Cancer 11, 644–656.
Verstraeten, N., Fauvart, M., Versees, W., Michiels, J., 2011. The universally conserved prokaryotic GTPases. Microbiol Mol Biol Rev 75, 507–42, second and third pages of table of contents.
Wallace, D.C., 2012. Mitochondria and cancer. Nat. Rev. Cancer 12, 685–698.
Wang, Z., Choi, S., Lee, J., Huang, Y.T., Chen, F., Zhao, Y., et al., 2015. Mitochondrial variations in non-small cell lung cancer (NSCLC) survival. Cancer Inform. 14, 1–9.
Warburg, O., 1956. On the origin of cancer cells. Science 123, 309–314.
Wu, S., Wan, P., Li, J., Li, D., Zhu, Y., He, F., 2006. Multi-modality of pI distribution in whole proteome. Proteomics 6, 449–455.
Wurth, L., 2012. Versatility of RNA-binding proteins in cancer. Comp. Funct. Genomics 2012, 178525.
Xu, Y., Johansson, M., Karlsson, A., 2008. Human UMP-CMP kinase 2, a novel nucleoside monophosphate kinase localized in mitochondria. J. Biol. Chem. 283, 1563–1571.
Yang, J., Hooper, W.C., Phillips, D.J., Tondella, M.L., Talkington, D.F., 2003. Induction of proinflammatory cytokines in human lung epithelial cells during Chlamydia pneumoniae infection. Infect. Immun. 71, 614–620.
Yoo, D.G., Song, Y.J., Cho, E.J., Lee, S.K., Park, J.B., Yu, J.H., et al., 2008. Alteration of APE1/ref-1 expression in non-small cell lung cancer: the implications of impaired extracellular superoxide dismutase and catalase antioxidant systems. Lung Cancer 60, 277–284.
Zhan, P., Suo, L.J., Qian, Q., Shen, X.K., Qiu, L.X., Yu, L.K., et al., 2011. Chlamydia pneumoniae infection and lung cancer risk: a meta-analysis. Eur. J. Cancer 47, 742–747.
Zhang, L., Pelech, S., Uitto, V.J., 2004. Bacterial GroEL-like heat shock protein 60 protects epithelial cells from stress-induced death through activation of ERK and inhibition of caspase 3. Exp. Cell Res. 292, 231–240.
Zhang, L., Pelech, S.L., Mayrand, D., Grenier, D., Heino, J., Uitto, V.J., 2001. Bacterial heat shock protein-60 increases epithelial cell proliferation through the ERK1/2 MAP kinases. Exp. Cell Res. 266, 11–20.
Zhao, L., Wang, D.L., Liu, Y., Chen, S., Sun, F.L., 2013. Histone acetyltransferase hMOF promotes S phase entry and tumorigenesis in lung cancer. Cell. Signal. 25, 1689–1698.
Zhao, R., Yan, Q., Lv, J., Huang, H., Zheng, W., Zhang, B., et al., 2012. CHD5, a tumor suppressor that is epigenetically silenced in lung cancer. Lung Cancer 76, 324–331.
Zugel, U., Kaufmann, S.H., 1999. Role of heat shock proteins in protection from and pathogenesis of infectious diseases. Clin. Microbiol. Rev. 12, 19–39.