Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 6 Issue 9 September 2022

Research Article

Immunohistochemical Evaluation of Interleukin 2 (IL2) And MKI67 on Buccal Samples from Local Residents in Nlng Ogbunagha Yenagoa (Yelga), Bayelsa State

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Received: April 11, 2022

Published: August 05, 2022

Abstract

Immunohistochemistry (IHC) is an important application of monoclonal as well as polyclonal antibodies to determine the tissue distribution of an antigen of interest in health and disease. IHC is widely used for diagnosis of cancers; specific tumor antigens are expressed de novo or up-regulated in certain cancers. A buccal swab, also known as buccal smear, is a way to collect from the cells on the inside of a person's cheek (buccal cavity). Buccal swabs are a relatively non-invasive way to collect buccal samples for testing. The expression of MKi67 is strongly associated with tumor cell proliferation and growth, and is widely used in routine pathological investigation as a proliferation marker. Interleukin-2 (IL-2), also known as aldesleukin or proleukin. It is a type of cytokine signaling molecule in the immune system which is a naturally occurring protein that is produced by a specific type of white blood cell, (T lymphocyte). This study evaluated the Immunohistochemistry (IHC) effect on (IL2) and MKi67 on Buccal samples from local residents in Nigeria Liquefied Natural Gas (NLNG), Obunagha-Yenagoa, Bayelsa State. A total of one hundred (100) buccal samples were collected for this study, 50 (50%) for the test and 50 (50%) being for control. Result analysis revealed that there were no abnormal expression of the MKi67 and interleukin 2 (IL2) in NLNG Obunagha residents. The samples collected and studied showed 0% of cancer predisposition and immunomodulation. Collaborative studies should be done in a wider range that cuts across Nigerian oil companies and also residents in those areas. Other companies involved in any form of burning and filling stations should be cited far away from residential areas so as to prevent crude oil toxicity and pollution.

Keywords: Crude Oil; NLNG; Immunohistochemistry; Buccal Smear

Introduction

Crude oil has been known to cause numerous and significant health issues for children, pregnant mothers, and adults. Exposure occurs via inhalation, ingestion (of liquid droplets in the air), and direct skin contact. Some of the health effects will be long-term sequelae and may last a lifetime or affect future generations. Crude oil is a known teratogen and can cause birth defects and changes in fetal development. The target organs for crude oil are the hematopoietic (blood forming) system, lymphatic system, nervous system, and reproductive system. The Benzene component is a known carcinogen [2].

[32], in his work reported that any chemical that can cross the placenta influences the development of the embryo and fetus. This is particularly true at the time when cells are dividing and

differentiating into specific tissues of the nervous, circulatory, and immune systems. According to the World Health Organization, the embryo, fetus, or child is particularly sensitive to even minute concentrations of toxic chemicals. Crude oil and its components are known teratogens and cause birth defects, changes in fetal development, and decreased fetal survival. Inhalation of fumes from crude oil are known to cause chemical pneumonia, irritation of the nose, throat, and lungs, headache, dizziness, drowsiness, loss of coordination, fatigue, nausea, and labored breathing. Chronic exposure can result in irregular heartbeats, convulsions, and coma.

Specifically, Crude Oil (CAS #8002-05-9) contains Benzene, Butane, N-Hexane, Isopentane, Pentane, and Stoddard Solvent. Benzene is a known human carcinogen and is identified by NTP, OSHA, and IARC as a Group 1 carcinogen. Chronic inhalation of minute levels of benzene causes leukemia and other types of cancers [27].

Acute contact, via inhalation and skin, with small amounts of light crude oil and dispersants cause transitory respiratory, vomiting, diarrhea, and skin reactions. However, long-term exposure, which can be a matter of days or weeks, can cause central nervous system problems, or do damage to blood and organs such as kidneys or livers, according to the Centers for Disease Control and Prevention. There is also a significant increase in the risk of cancer [1].

Crude oil is not readily biodegradable and the effects of exposure to this toxin will be felt from generation to generation. Children and pregnant mothers are at significant risk. All exposures, no matter how seemingly insignificant, may prove to be consequential. What may seem to be a relatively trivial exposure in a healthy individual may potentially prove catastrophic, and the consequences of both acute and chronic exposures to crude oil may take years, even decades, to fully reveal the array of disease and morbidity than will result from exposure to this substance.

Molecular markers have been extensively investigated with a view to providing early and accurate information on long-term outcome and prediction of response to treatment of early cancer. Proliferation is a key feature of the progression of tumors and is now widely estimated by the immunohistochemical assessment of the nuclear antigen Ki-67. The expression of Ki-67 correlates with other measurements of proliferation, including S-phase and bromodeoxyuridine uptake. High Ki-67 is a sign of poor prognosis associated with a good chance of clinical response to chemotherapy [15].

Since its discovery in 1985 the soluble interleukin-2 receptor (sIL-2R, sTAC, sCD25) has become a clinically valuable tool for several diseases. It is regarded as a disease activity marker in sarcoidosis, but increased serum levels have been also observed in other autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis. In addition, sIL-2R is elevated in several neoplastic disorders, and it appears useful in estimating survival and monitoring therapy in malignancies like malignant melanoma or nasopharyngeal carcinoma. The interleukin-2 receptor is a heterotrimeric transmembrane protein that is upregulated on activated T cells, and high sIL-2r levels are found in hemophagocytic syndromes, lymphoma, autoimmune lymphoproliferative syndrome, and other conditions associated with T-cell activation. These biomarkers; interleukin 2 (IL2) and Mki67 can be used to determine the predisposition of local residents to various diseases [31].

Materials and Methods

Study area

This study was carried out in NLNG, Obunagha, Yenagoa, Bayelsa State, South-South, Nigeria. Bayelsa is located within Lat. 4151N and Lat. 523 South and Long. 5221 adnd 651 East of the equator, bounded by the Atlantic Ocean by the South Coast, Nigeria. The state is the second largest producer of crude oil in Nigeria and has the largest gas reserve and oil well. Her major occupation is farming and fishing.

Sample collection

A total of 50 buccal smear samples were collected from local residents in NLNG, Obunagha, Yenagoa, Bayelsa State, South-South, Nigeria and A total of 50 control samples were also collected. The samples were sent to the Pathology Department, Histopathology Unit, Obafemi Awolowo University Teaching Hospital, Ile-Ife, Osun State. Samples used in this study were collected in November, 2021.

Materials used

Buccal smear samples were obtained from the local residents from NLNG Obunagha, Yenagoa, Bayelsa state. The samples were collected with the aid of a wooden spatula.

Methodology

The smears were fixed in 95% ethanol for 1hr. it was then rinse in tap water, followed by distilled water after which it the smears were circled with hydrophobic pen to prevent free flow of reagent off the smear. On the other hand, antigen from the control which was formalin fixed paraffin embedded tissue sectioned were retrieved using pressure pot antigen retrieval method before the sections were circled with hydrophobic pen. Both control and test were then washed in 10% phosphate buffer saline (PBS). Slides were drained, two Drops of hydrogen peroxide block was added to cover the section and incubate for 10 minutes to block endogenous perioxidases. Washed times in PBS, Protein Block was added and incubate for 10 minutes at room temperature to block nonspecific background staining. Slides were washed one time in PBS, one primary diluted antibody (MKi67 and IL-2) for a slide was added and incubated at room temperature for 1hr. Washed in PBS, complement was added, and incubated for 10 minutes at room temperature. Washed in PBS, HRP conjugate was added and incubated for 15 minutes at room temperature. Washed in PBS, DAB/Substrate was added to the tissue section and incubated for 7 minutes. Wash in PBS, stained with Haematoxylin for 2 minutes, blue in tap water for 3minutes, dehydrated, cleared and mounted with resineous mountant.

Observation

No rigid scoring system is in place for the antibodies used and most immunohistochemistry antibodies. Immunohistochemistry slides were reported based on the average percentage of brown staining cytoplasm and nuclei.

Statistical analysis

The statistical analysis of the data was done using SPSS software for Windows, Chi_Square test and patient test was used to check the significance of the data. P value less than 0.05 was defined as statistically significant for the data.

Results

A total of fifty (50) buccal smear samples and fifty (50) control samples in November, 2021 was included in this study. Out of 50 samples and 50 control samples, the result showed IL2-negative and MK167-negative. The result was represented in photomicrographs (figure 1 and 2).

	Frequency	Percent
Control	30	50
Test	30	50
Total	60	100

Table 1a: Demographic distribution of survey participants.

Figure 1: Graphical representation of survey participants.

	Minimum age	Maximum age	Mean age
Control	21	41	29.67 ± 6.88
Test	23	57	40.27 ± 8.92

Table 1b: Age analysis of survey participants.

	Control	Test
Occupation		
Business	00	18
Driver	00	04
Electrical engineer	00	02
Hair dresser	02	02
POS attendant	08	02
Student	20	00
Trader	00	02
TOTAL	30	30

Table 1c: Demographic distribution of survey participants based on occupation.

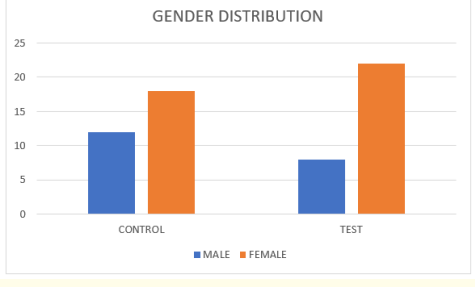

Figure 2: Gender distribution of survey participants.

	Control (%)	Test (%)	Total (%)
Age classification			
21 - 30 years	18 (30)	04 (6.7)	22 (36.7)
31 - 40 years	08 (13.3)	12 (20)	20 (33.3)
41 - 50 years	04 (6.7)	10 (16.7)	14 (23.4)
51 - 60 years	00 (00)	04 (6.7)	04 (6.7)
Total	30 (50)	30 (50)	60 (100)

Table 3: Age based demographics of survey participants.

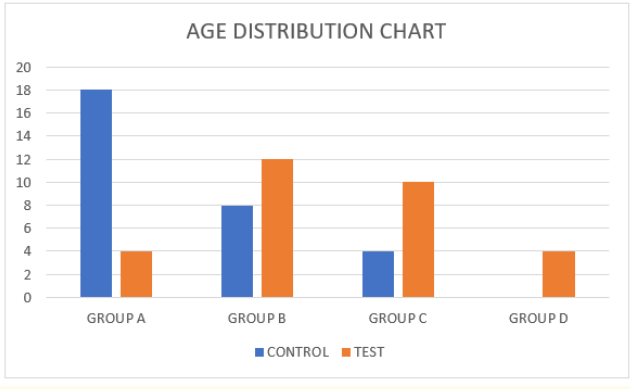

Figure 3: Age distribution of survey participants.

Key

Group A: 21 - 30 years

Group B: 31 - 40 years

Group C: 41 - 50 years

Group D: 51 - 60 years.

	Control (%)	Test (%)	Total (%)
Years of residence			
<5 years	21 (35)	10 (16.7)	31 (51.7)
5 - 10 years	08 (13.3)	16 (26.7)	24 (40)
11 - 15 years	01 (1.7)	04 (6.7)	05 (8.4)
>15 years	00 (00)	00 (00)	00 (00)
Total	30 (50)	30 (50)	60 (100)

Table 4: Demographic distribution of survey participants based on duration of residence in years.

	IL2		IL7	
	Positive	Negative	Positive	Negative
Control	00	30	00	30
Test	00	30	00	30

Table 5: Interleukin analysis of survey participants.

Plate 1: MKI67 Positive Control Tissue.

The slide show positive nuclei stained brown (arrow) by MKI67 antibody.

Plate 2: MKI67 Immunoreactivity on Non Smoker Buccal Smear.

The slide shows no immunoreactivity on the buccal smear from non smoker.

Plate 3: MKI67 Immunoreactivity on A Smoker Buccal Smear.

The smear shows no nucleus positively stained brown for MKi67 indicating negativity.

Plate 4: Positive Control Tissue for IL-2.

The slide depicts some cells nuclei and cytoplasm being positively stained brown (arrow) by interleukin 2.

Plate 5: IL-2 Immunoreactivity on Non Smoker Buccal Smear.

The slide shows no immunoreactivity on the buccal smear from non- smoker.

Plate 6: IL-2 Immunoreactivity on A Smoker's Buccal Smear.

The smear shows no nucleus positively stained brown for MKI67 indicating negativity.

Discussion

Molecular markers have been extensively investigated with a view to providing early and accurate information on long-term outcome and prediction of response to treatment of early cancer. Proliferation is a key feature of the progression of tumors and is now widely estimated by the immunohistochemical assessment of the nuclear antigen Ki-67. High Ki-67 is a sign of poor prognosis associated with a good chance of clinical response to chemotherapy [15]. The interleukin-2 receptor is a protein that is upregulated on activated T cells, and high sIL-2r levels are found in hemophagocytic syndromes, lymphoma, autoimmune lymphoproliferative syndrome, and other conditions associated with T-cell activation. These biomarkers; interleukin 2 (IL2) and Mki67 can be used to determine the predisposition of local residents to various diseases, [31] associated with long term exposure to crude oil pollution.

A total of 50 buccal smear samples were collected from local residents in NLNG, Obunagha, Yenagoa, Bayelsa State, South-South, Nigeria and a total of 50 control samples were also collected. All 100 samples gotten for immunohistochemical evaluation of buccal smears where shown to be negative to the presence of cancerous lesions.

In a study that was conducted to investigate the effect of petrol vapor containing benzene on inflammatory and immune markers of human, besides its effect on hematological and immunological parameters. It was found that the level of IL-6 as a proinflammatory marker was significantly increased in the workers group compared to the non exposed group. This is due to different pathological pathways which may be induced as a result of continuous exposure to the pollutants in the air. Despite that the effect of benzene exposure on human health has been proven, the mechanism of benzene toxicity is still not completely understood [22]. However, the toxicity may act through different pathways. These pathological pathways result in releasing different mediators and inflammatory molecules including cytokines. Their release by T-cells and macrophages may induce a series of reactions that result in systemic inflammation and play a significant role in immune response. The ability of benzene to result in an imbalance in the immune system was concluded by other studies, and that

benzene may damage the lymphokines producing system, which regulates both hematopoiesis and immunity [13]. A previous study has concluded that specific cytokines producing cells were highly sensitive to the toxic effect of benzene; therefore, the immune system would become imbalanced, and that benzene can damage the system responsible for producing lymphokines and inhibit the immune function and hematopoiesis.

Conclusion

In this study, result analysis revealed that there was no abnormal expression of the MKI 67 and interleukin 2 (IL2) in NLNG Obunagha residents. The samples received showed 0% of cancer predisposition and immunomodulation. Further workup and studies should be done with regards to the predisposition of workers and residents in oil companies which would in turn help to educate individuals on the dangers associated with exposure to crude oil pollution. Although it was found that most occupants of NLNG Obunagha were found to be smokes with some having signs of ulceration in their buccal cavities due to excess smoking. Furthermore, the expression varies on smokers, non smokers and age groups.

Recommendation

Further studies should be done in a wider range that cuts across Nigerian oil companies and also residents in those areas. Also companies involved in any form of singeing and filling stations should be cited far away from residential areas so as to prevent crude oil toxicity and pollution.

Funding Support

This research did not receive any financial support from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgements

The authors would like to thank the residents of NLNG Obunagha for their cooperation and compliance. Our sincere appreciation goes to the research assistants Atua, Felix Azibagiri and Odisi, Nelson Elaye for their relentless effort in carrying out the field survey and collection of smears from residents of NLNG Obunagha for this work. The authors are grateful to Mr. Okiyo, Emmanuel Torugbene and Mr. Agu, Charles for providing the logistical support in meeting with the residents of NLNG Obunagha. The authors are also appreciative of the statistical support received from Mr. Odumosu, Newton.

Bibliography

1. Abdrabouh A., *et al*. "Health assessment approach for evaluating hematologic and immune toxicity of prolonged gasoline inhalation in fuel station workers at Mansoura city, Egypt". *Journal of Applied Environmental and Biological Sciences* 7.8 (2017): 30-36.

2. Abou-ElWafa H., *et al*. "Some biochemical and hematological parameters among petrol station attendants: a comparative study". *BioMedical Research International* 6.3 (2015): 6-12.

3. Agrawal P., *et al*. "Air pollution and cytokines". *Journal of Medical Academics* 1.1 (2018): 43-49.

4. Barak V., *et al*. "Serum inflammatory cytokines, complement components, and soluble interleukin 2 receptor in primary biliary cirrhosis". *Journal of Autoimmunity* 33.3-4 (2009): 178-182.

5. Bien E and Balcerska A. "Serum soluble interleukin 2 receptor alpha in human cancer of adults and children: a review". *Biomarkers* 13.1 (2008): 1-26.

6. Brown D and Gatter K. "Ki67 protein: the immaculate deception?" *Histopathology* 40 (2002): 2-11.

7. Chan J., *et al*. "Antisense oligonucleotides: from design to therapeutic application". *Clinical and Experimental Pharmacology and Physiology* 33 (2006): 533-540.

8. Chen F., *et al*. "IRF1 suppresses Ki67 promoter activity through interfering with Sp1 activation". *Tumour Biology* 33 (2012): 2217-2225.

9. Chen J., *et al*. "A novel molecular grading model: combination of Ki67 and VEGF in predicting tumor recurrence and progression in non-invasive urothelial bladder cancer". *Asian Pacific Journal Cancer Prevention* 13 (2012): 2229-2234.

10. De Aguiar H., *et al*. "Labeling index in pituitary adenomas evaluated bymeans of MIB 1: is there a prognostic role? A critical review". *Neurology Research* 32 (2010): 1060-1071.

11. Dobreva G., *et al*. "Proinflammatory and anti-inflammatory cytokines in adolescents from Southeast Bulgarian cities with different levels of air pollution". *Toxicology and Industrial Health* 31.12 (2015): 1210-1217.

12. Fenga C., *et al*. "Immunological effects of occupational exposure to lead". *Molecular Medicine Reports* 15.5 (2017): 3355-3360.

13. Gillis B., *et al*. "Identification of human cell responses to benzene and benzene metabolites". *Genomics* 90.3 (2007): 324-333.

14. Hammond ME., *et al*. "American Society of Clinical Oncology/ College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer". *Journal of Clinical Oncology* r 28.16 (2010): 2784-2795.

15. Hegazy A., *et al*. "Role of Ki 67, P53 and Bcl 2 in Advanced Colorectal Carcinoma". *Academic Journal of Cancer Research* 7.3 (2014): 168-172.

16. Heymann F., *et al*. "Monocytes and macrophages as cellular targets in liver fibrosis". *Inflammation and Allergy Drug Targets* 8.4 (2009): 307-318.

17. Hoogerbrugge N., *et al*. "Tumour examination to detect hereditarycolorectal cancer". *Ned Tijdschr Geneeskd* 156 (2012): A4982.

18. Hu H., *et al*. "Clinical significance of Smac and Ki-67 expression in pancreatic cancer". *Hepato-gastroenterology* 59 (2012): 2640-2643.

19. Ishihara M., *et al*. "Retrospective analysis of risk factors for central nervous system metastases in operable breast cancer: effects of biologic subtype and Ki67 over expression on survival". *Oncology* 84 (2013): 135-140.

20. Junghans R and Waldmann T. "Metabolism of Tac (IL2Ralpha): physiology of cell surface shedding and renal catabolism, and suppression of catabolism by antibody binding". *Journal of Experimental Medicine* 183.4 (1996): 1587-1602.

21. Karlmark K., *et al*. "Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promoteshepatic fibrosis". *Hepatology* 50.1 (2009): 261-274.

22. Kasemy Z., *et al*. "Environmental and health effects of benzene exposure among Egyptian taxi drivers". *Journal of Environmental and Public Health* 201 (2019): 6-26.

23. Kim B., *et al*. "Usefulness of Ki67 (MIB1) immunostaining in the diagnosis of pulmonary sclerosing hemangiomas". *Acta Pathologica, Microbiologica, et Immunologica Scandinavica* 121 (2013): 105-110.

24. Kimura T., *et al*. "Clinical significance of MUC1 and E-cadherin expression, cellular proliferation, and angiogenesis at the deepest invasive portion of colorectal cancer". *International Journal of Oncology* 16 (2000): 55-64.

25. Li L., *et al*. "A small molecule Smac mimic potentiates TRAIL and TNFalpha mediated cell death". *Science* 305 (2004): 1471-1474.

26. Liu J., *et al*. "Effects of G250 promoter controlled conditionally replicative adenovirus expressing Ki67 siRNA on renal cancer cell". *Cancer Science* 103 (2012): 1880-1888.

27. Machowska M., *et al*. "Nuclear location of tumor suppressor protein maspin inhibits proliferation of breast cancer cells without affecting proliferation of normal epithelial cells". *BMC Cancer* 14 (2014): 142-201.

28. Mahmoud H., *et al*. "Phenotype analysis of lymphocytes in workers with chronic benzene exposure". *Egyptian Journal of Haematology* 42.4 (2017): 161-168.

29. McKenzie S and Williams L. "Clinical Laboratory Hematology". Pearson, London, UK, 3rd edition (2014): 114.

30. Moore C., *et al*. "Short hairpin RNA (shRNA): design, delivery, and assessment of gene knockdown". *Methods in Molecular Biology* 629 (2010): 141-158.

31. Nabi U., *et al*. "Ki 67 proliferating index and histological grade, type and stage of colorectal carcinoma". *Ayub Medical Colledge Jourrnal Abbottabad* 20 (2008): 44-48.

32. Ndubuisi E. "Haematological indicators of exposure to petroleum products in petroleum refining and distribution industry workers in Nigeria". *Journal of Clinical Toxicology* 6.1 (2016): 1-13.

33. Nielsen P., *et al*. "Automated quantification of MART1 verified Ki67 indices by digital image analysis in melanocytic lesions". *Archives of Pathology and Laboratory Medicine* 136 (2012): 627-634.

34. Panteva M., *et al*. "Direct observations of shifts in the β sheet register of a protein peptide complex using explicit solvent simulations". *Biophysics Journal* 100 (2011): 50-52.

35. Park Y., *et al*. "Immunohistochemical analysis for therapeutic targets and prognostic markers in low-grade endometrial stromal sarcoma". *International Journal of Gynecology and Cancer* 23 (2013): 81-89.

36. Pesatori A., *et al*. "Early effects of low benzene exposure on blood cell counts in Bulgarian petrochemical workers". *La Medicina Del Lavoro* 100.2 (2009): 83-90.

37. Romagnani S. "T-cell subsets ('1 versus '2)". *Annals of Allergy, Asthma and Immunology* 85.1 (2000): 9-21.

38. Rubin L., *et al*. "Soluble interleukin 2 receptors are released from activated human lymphoid cells *in vitro*". *Journal of Immunology* 135.5 (1985): 3172-3177.

39. Scholzen T and Gerdes J. "The Ki67 protein: from the known and the unknown". *Journal of Cell Physiology* 182 (2000): 311-322.

40. Seidler E. "Elevated circulating soluble interleukin 2 receptor in patients with chronic liver diseases is associated with non-classical monocytes". *BMC Gastroenterology* 12 (2012): 38.

41. Seidler S., *et al*. "Age dependent alterations of monocyte subsets and monocyte-related chemokine pathways in healthy adults". *BMC Immunology* 11 (2010): 30.

42. Snyder R. "Leukemia and benzene". *International Journal of Environmental Research and Public Health* 9.8 (2012): 2875-2893.

43. Tacke F., *et al*. "Inflammatory pathways in liver homeostasis and liver injury". *Clinical Revolution in Allergy and Immunology* 36.1 (2009): 4-12.

44. Tvedskov T. "Staging of women with breast cancer after introduction of sentinel node guided axillary dissection". *Danish Medical Journal* 59 (2012): 4475.

45. Vogt N and Klapper W. "Variability in morphology and cell proliferation in sequential biopsies of mantle cell lymphoma at diagnosis and relapse: clinical correlation and insights into disease progression". *Histopathology* 62 (2013): 334-342.

46. Yerushalmi R., *et al*. "Ki67 in breast cancer: prognostic and predictive potential". *Lancet Oncology* 11 (2010): 174-183.

47. Zhang B. "Neoadjuvant endocrine therapy for postmenopausal estrogen receptor positive patients with breast cancer". *Zhonghua Zhong Liu Za Zhi* 33 (2011): 241-244.

48. Zhang P., *et al*. "Versatile photosensitizers for photodynamic therapy at infrared excitation". *Journal of the American Chemical Society* 129 (2007): 4526 -4527.

49. Zheng J., *et al*. "Knockdown of Ki-67 by small interfering RNA leads to inhibition of proliferation and induction of apoptosis in human renal carcinoma cells". *Life Science* 78 (2006): 724-729.

50. Zheng J., *et al*. "Inhibition of renal cancer cell growth *in vitro* and *in vivo* with oncolytic adenovirus armed short hairpin RNA targeting Ki-67 encoding mRNA". *Cancer Gene Therapy* 16 (2009): 20-32.

51. Zimmermann H and Tacke F. "Modification of Chemokine Pathways and Immune Cell Infiltration as a Novel Therapeutic Approach in Liver Inflammation and Fibrosis". *Inflammatory Allergy and Drug Targets* 10.6 (2011): 509-536.

52. Zimmermann H., *et al*. "Functional contribution of elevated circulating and hepatic nonclassical CD14CD16 monocytes to inflammation and human liver fibrosis". *PLoS One* 5.6 (2010): 11049.