Chapter 5

CONCLUSIONS AND FUTURE RESEARCH PERSPECTIVES

5.1. HIGHLIGHTS OF THE RESEARCH OUTCOME

A radiation-induced thymic lymphoma (TL) model was developed in Swiss mice to study the mechanism and modification of radiation carcinogenesis. Exposure of 3 Gy total body γ-irradiation (TBI) induced TL tumor in Swiss mice. The incidence of the TL was dependent on the age at TBI and gender of the mice. It was intended to characterize the tumor by histopathological typing using the tumor model. Histopathology analysis of the thymus on 120 d post TBI revealed loss of corticomedullary delineation, conspicuous mitotic activities and apoptosis with lymphocytes simulating lymphoblasts. The overall histopathological characteristics indicated features of a high grade non Hodgkin's lymphoma.

Gene microarray analysis performed in TL tumor tissues revealed differential expression of a significant number of genes. Results also suggested involvement of several biological processes involving apoptosis, cell cycle regulation, immunity and defense, antioxidant enzymes, cell surface proteins, calcium signaling and oncogenesis processes during radiation induced TL development.

The role of antioxidant enzymes and oxidative stress in incidence and pathogenesis of the tumor has been studied. Studies showed higher SOD and catalase activities in thymic tissue than in serum of irradiated animals. Generation of ROS and induction of apoptosis in thymocytes of mice exposed to increasing doses (1-5 Gy) of TBI increased in a dose dependent manner. Generation of ROS was 2 and 6.5 folds higher with 3 and 5 Gy TBI respectively compared to that of 1 Gy. Even the level of apoptosis was
increased 3 to 4.5 times with the exposure of 3 to 5 Gy TBI.

Modification of the TL incidence was studied following administration of dietary antioxidants and low doses of radiation. Feeding of dietary antioxidants, vit C (600 mg/kg), vit E (25 mg/kg) or eugenol (300 mg/kg) gave about 21-28% protection, whereas feeding of curcumin (1% in diet) gave up to 58% protection to the mice in terms of induction of radiation-induced TL incidence. Curcumin feeding following transplantation of TL also delayed the appearance of the tumor by 66.67% up to 10 d in the host mice. Moreover, the period of administration of dietary curcumin was observed to be an important determinant in elucidating its role in suppression of transplanted tumor. Treatment of mice with relatively low-dose TBI of 10, 30 and 50 cGy 6 h prior to the acute high dose (3 Gy) drastically reduced the incidence TL by 22, 66 and 22% respectively compared to control receiving only 3 Gy. The lower TL incidence in the group of mice receiving 30 cGy priming dose is correlated with the findings of higher catalase activities and lower level of SOD in the serum.

After characterization of the TL and its modification with dietary antioxidants and low dose radiation, novel porphyrin derivatives were explored for radioisotope based imaging and therapy of the tumor. Porphyrin derivatives, 5,10,15,20-tetrakis[3,4-bis(carboxymethyleneoxy) phenyl] porphyrin labeled with ^{188}\text{Re} (^{188}\text{Re}-\text{DHBEPH}), and meso-5,10,15,20-tetrakis[4-carboxymethyleneoxy-phenyl]porphyrin labeled with ^{177}\text{Lu} (^{177}\text{Lu}-labeled porphyrin-BFCA) were used for imaging and targeted radiotherapy agents in transplanted tumor. Serial scintigraphic images recorded using a single head digital gamma camera (SPECT) system exhibited selective localization of the ^{177}\text{Lu}-labeled porphyrin-BFCA in the tumor tissues. The significant uptake of the radiolabeled conjugate by the tumor tissues revealed that the agent could be used for diagnosis as well as pre-therapy imaging of tumor. After establishing the tumor imaging protocol, the labeled complexes were evaluated for understanding biological behavior a primary
step towards determining the suitability of using the complexes in targeted radionuclide therapy. Biokinetic studies of $^{188}$Re–DHBEPH radiochemical carried out in mice bearing fibrosarcoma as well as TL tumors was quite promising (>2% IA/g of tumor at 24 h p.i.). Retention of the $^{177}$Lu-labeled porphyrin-BFCA conjugate showed fairly high tumor to muscle ratio (26.50) in fibrosarcoma tumor tissue in mice at 48 h p.i. The radiolabeled conjugates were evaluated for their efficacy in regressing the fibrosarcoma and TL tumor with a view to develop as possible agents for targeted radionuclide therapy. $^{188}$Re–DHBEPH (1.5 mCi) treatment could effectively regress the volume of fibrosarcoma (32.86%) and TL (50.75%) tumor. Reduction in volume of the tumors due to $^{177}$Lu-labeled porphyrin-BFCA treatment (1.5 mCi) was comparable to $^{188}$Re–DHBEPH treatment. However, tumor volumes were substantially regressed (51% in fibrosarcoma and 76 % in case of TL) with the increasing dose (2 mCi x 2 doses) of $^{177}$Lu-labeled porphyrin-BFCA conjugate. The increased in average DT due to $^{188}$Re–DHBEPH (1.5 mCi) treatment was marginal (2.47±0.28 d as compared to 2.06±0.18 d in control) in fibrosarcoma tumor and very conspicuous (2.57±0.22 d as against 1.90±0.08 d in control) in TL bearing animals. Treatment of the tumor with $^{177}$Lu-labeled porphyrin-BFCA (2.0 mCi) increased the average DT 1.5 fold in fibrosarcoma and >2.5 fold in TL tumor. The decrease in SGR was marginal (0.28±0.03 %/d vs 0.34±0.03 %/d in control) in fibrosarcoma tumor and was marked (0.27±0.02 %/d vs 0.36±0.02 %/d in control) in the$^{188}$Re–DHBEPH (1.5 mCi) treated animals. The decrease in average SGR due to $^{177}$Lu-labeled porphyrin-BFCA treatment was comparable to that of $^{188}$Re–DHBEPH for both the tumor types with lower (1.0 and 1.5 mCi) dose and was significant (> 1.5 fold for fibrosarcoma and >2 fold for TL) at higher (2 mCi) dose. The radionuclide labeled conjugates, $^{188}$Re–DHBEPH, and $^{177}$Lu-labeled porphyrin-BFCA were found effective in terms of pre-therapy imaging and regression of tumor.

Following the successful demonstration of the novel porphyrin based targeted tumor therapy in the experimental tumor models, the research work
was further extended to study the efficacy of certain antineoplastic drugs/radiation in less toxic combinational treatment strategy. Radio and chemosensitivity of the TL tumor was investigated to demonstrate cytotoxic potential of radiation and the antineoplastic drug the TL cells. Radiosensitivity response of the tumor cells evaluated in terms of appearance and growth delay of transplanted tumor revealed a partial response at 12 and 16 Gy and complete response at 20 Gy radiation. Similarly antineoplastic drug doxorubicin (0.25 mg) was found most effective compared to cyclophosphamide and vinblastin in combating the growth of TL tumor. For enhancing the radio-cytotoxicity of the cancers that are crucial for surgical manipulation or conventional radiotherapy, an effective therapeutic protocol was developed using radio-electro-chemotherapy on the TL tumor model. Radiation (3 Gy) treatment decreased the viability of the tumor cells by about 7%, the decrease was 2 fold higher with the EP (2.0 kV/cm) and combination of both the treatment could decrease the viability by > 4 fold. Antineoplastic drug, doxorubicin (10 μg) treatment alone decreased the cell viability by 16.23 % and combination of the drug with EP brought the viability drastically (> 4 fold).

These results were further validated in in vivo tumor model. The tumor growth kinetic studies, showed about 48 % reduction in volume of TL following treatment with radiation (2 Gy), doxorubicin and EP which was reflected by the increase (1.18 ± 0.14 d) in the average tumor DT in the combination treatment group compared to that of control animals (1.03 ± 0.06 d) on 7 d. The efficacy of the combination treatment of radio-electro-chemotherapy on the regression of the TL tumor was established by the marked delay (0.59 ± 0.08 %/d) in the average SGR against the corresponding value (0.68 ± 0.04 %/d) in untreated control.
5.2. FUTURE RESEARCH PERSPECTIVES

The results obtained in this thesis opens up many new areas of research for future which are listed below in brief:

1. To establish the role of sex hormone in the mechanism of radiation-induced thymic lymphomagenesis.

2. To establish the role of genes/proteins associated with apoptotic pathways, oxidative metabolism and immune system in the mechanism of radiation-induced TL incidence.

3. To investigate the role of genetic and physiological factors governing the individual susceptibility to the TL incidence.

4. To study the cellular and molecular pathways associated with apoptotic and oxidative damage in modification of TL incidence by antioxidants and low-dose radiation.

5. Mechanism of protection against TL incidence by curcumin in radiation exposed animals.

6. To synthesize/develop newer porphyrin derivatives for better tumor localizing ability.

7. To evaluate the efficacy of the porphyrin labeled radionuclides in appropriate human xenograft.

8. To study antitumor mechanism of the therapeutic β-emmiting radionuclides in vivo with reference to the induction of apoptotic and bystander response by the host.

9. To study the effect of antineoplastic drug and radiation in combination with other EP conditions in appropriate tumor models.