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## CHAPTER 1: INTRODUCTION AND REVIEW OF LITERATURE 1-45

- **Cancer**
  - Hallmarks of Cancer
  - Epidemiology of Cancer: Global and Indian Scenario

- **Oral Squamous Cell Carcinoma: The Most Avoidable Cancer**
  - Etiology of Oral Squamous Cell Carcinoma
  - Diagnostic Modalities for Early Detection of Oral Cancer
  - Conventional Therapeutic Strategies for Oral Cancer
  - TNM Classification of OSCC
  - Major Failures in Diagnosis & Therapeutic Modalities of Oral Carcinoma

- **Cancer Stem Cells**
  - The Cancer Stem Cell Models: Stochastic v/s Hierarchic
  - Cancer Stem Cell Markers In OSCC
  - Epigenetic Regulatory Mechanisms Modulating OSCC CSC Signatures
  - Role Of Epithelial - Mesenchymal Transition In Cancer Stem Cells
  - Signaling Pathways Modulating Cancer Stem Cells
  - The Nicotine Link In Cancer Stem Cell Prototype

- **Circulating Tumor Cells: Predictors of Metastasis and Recurrence**
  - Technologies For Identification Of Circulating Tumor Cells
  - Circulating Tumor Cells in HNSCC: An Unexplored Arena
  - EMT in CTCs: Phenomena Of Self Seeding (Recurrence) or Identifying The Soil (Metastasis)
  - Circulating Tumor Stem Like Cells

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CHAPTER 2: TO EXPLORE THE MUTATIONAL SPECTRUMS IN TOBACCO HABITUATED & NON-HABITUATED SUBJECTS OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

Rationale of the Study

Methodology

- Collection of OSCC Patient Derived Tissue and Matched Peripheral Blood Samples
- DNA Extraction and Sequencing
- Data Analysis

Results

- Variant Analysis
- Chromosomal Distribution of Variants
- Detection of Cancer Driver Variants and Genes
- Assessment of Mutation Distribution at Gene Level
- String Protein-Protein Interaction and Pathway Analysis of Common Variants

Discussion

CHAPTER 3: TO EXPLORE THE ROLE OF MIRNAS AND TUMOR MICROENVIRONMENT IN MODULATING CSCS IN A PATHWAY DEPENDENT MANNER

Rationale of the Study

Methodology

- OSCC Tissues Acquisition and Preparation
- Cell Culture and Immuno-Magnetic Cell Sorting Of Cancer Stem Cells
- Sphere Forming Assay
- Drug Cytotoxicity Assay
- Pathway Prediction Analysis and Quantification of Identified Cscs Markers and miRNA Modulators in OSCC Samples
Bioplex Cytokine Profiling Of OSCC Samples

**Results**

- Identification and Characterization of Cancer Stem Like Cells from Primary Tissue of OSCC Patients
- Increased Self Renewal Capability and Tumorigenic Potential of CD44+ Cells
- CD44+ Cancer Stem Like Cell Population Demonstrated Chemo Resistance
- Gene Expression Profile of CSC Markers in CD44+ Cells and Their Significance in OSCC Patients
- Identification and Expression Profiling Of miRNA Modulators Regulating These CSC Markers in Pathway Dependant Manner
- Evaluating The Cytokine Network in OSCC Patients and Their Impinging Role in Regulating miRNA Mediated CSC Pathway

**Discussion**

**CHAPTER 4: TO ISOLATE & CHARACTERIZE THE CIRCULATORY TUMOR STEM CELL POPULATION AND DEMONSTRATE ITS CRITICAL ROLE IN PROGNOSIS OF OSCC PATIENTS**

**Rationale of the Study**

**Methodology**

- OSCC Tissues Acquisition and Preparation
- Cell Culture and Immuno-Magnetic Cell Sorting Of Cancer Stem Cells
- Flow Cytometry
- Sphere Forming Assay
- Drug Cytotoxicity Assay
- Quantitative Gene Expression of Pertinent CSC markers
- Statistical Analysis

**Results**

- Identification and Characterization of Circulating Tumor Stem like
Cells in Peripheral Blood of OSCC

- CD44+ cells demonstrated dose-dependent drug resistance
- Difference in Expression Patterns of Pertinent CSC Markers in Circulating CD44+ and CD44- Subpopulation
- Association of Increased Expression Patterns of CD44s, CD44v6 and Nanog with Anatomic Location, Loco-regional Aggressiveness and Recurrence in OSCC

Discussion

CHAPTER 5: TO UNDERSTAND THE SIGNIFICANCE OF TARGETING CD44v6 ON CELL PROLIFERATION, INVASION, APOPTOSIS AND CHEMO SENSITIVITY IN A PATHWAY DEPENDENT MANNER

Rationale of the Study

Methodology

- Cell Lines and Cell Culture
- siRNA Construction and Transfection
- Western Blotting
- Sphere Forming Assay
- Drug Cytotoxicity Assay
- Cell Migration Assay
- Flow Cytometry Analysis

Results

- Distinct Expressions of CD44v6 in Cell Lines and Immuno-Magnetic Sorting of CD44+ Cancer Stem Like Cell Population
- CD44v6 siRNA Mediated Transfection in HCT116-CD44+ and MDA-MB-231-CD44+ Cells and Altering Various Gene Expression Patterns
- Suppression of CD44v6 Enhances Chemosensitivity of HCT116-CD44+ and MDA-MB-231-CD44+ Cells
• Effects of CD44v6 Downregulation on Cell Cycle Analysis and Apoptosis
• Significance of CD44v6 on Activation of PI3K/AKT-MAPK Dual Signaling Pathways

Discussion

CHAPTER 6: TO SCREEN POTENTIAL LEAD COMPOUNDS AGAINST CD44v6: AN HOMOLOGY MODELING, DOCKING AND MOLECULAR DYNAMIC SIMULATION APPROACH

Rationale of the Study

Methodology

• Template Identification and Protein Model Preparation
• Model Validation
• Molecular Docking
• Molecular Dynamic Simulation

Results

• Template Identification and Protein Structure Prediction of CD44v6
• Lead Compound Identification by Virtual Molecular Docking
• Insight into Structural Perturbation of CD44v6 Bound with Potential Lead Compounds by Molecular Dynamic Simulation
• Interaction Profile Analysis of CD44v6 with HA and Its Potential Lead Compounds

Discussion

CHAPTER 7: SUMMARY AND CONCLUSION

REFERENCES

APPENDIX