GENERAL SUMMARY

The main observations of the present works are summarized as follows:

**Chapter 1: Identification of cytotoxic mediators associated with DHA-induced cell death in brain cancer cells, regulated differently in healthy primary astrocyte cultures.**

1. Treatment of C6 glioma and SH-SY5Y cell lines with 100 µM DHA for 24 h showed significant loss of cell viability as observed by using MTT assay. However, at this concentration, DHA showed no toxic effect on primary astrocytes cultures.

2. Such loss of cell viability in the neoplastic cells was due to apoptosis as evident from TUNEL assay, increased caspase activity and decreased expression of Bcl2.

3. High throughput proteomic approach identified six proteins in the cancer cells upon exposure to DHA which, unlike in the astrocytes, were differently altered, suggesting their putative contribution in causing apoptosis in cancer cells.

4. Of these, Annexin A2, calumenin, pyruvate kinase M2 isoform, 14-3-3δ were downregulated while aldo keto reductase-1B8 (AKR1B8) and glutathione–S-transferase P1 subunit (GSTP1) were upregulated.

5. Selective upregulation of PPARα and the MAP kinases, JNK and p38 as well as increased ROS production were observed in the cell lines upon exposure to DHA.

Overall, results of the present study suggest that DHA selectively induces apoptosis in the neural cell lines by regulating the expression of the above proteins to activate multiple apoptotic pathways which in association with excess ROS and activated MAPKs promote cell death.

**Chapter 2: Comparative studies on the molecular mechanism of TH or DHA-induced astrocytes differentiation.**

**Part 1: Identifying the key players during TH-induced differentiation of astrocytes**

1. ¹²⁵I-PIN binding to β-AR showed a significant decrease during 2-12 h, when 10 day old astrocytes culture were exposed to TH for a time period of 48 h. The decreased binding was due to a selective effect on β₂-AR.

2. The initial decrease in β₂-AR in membranes resulted in a concomitant increase in β₂-AR levels in the cytosol, suggesting that TH may induce endocytosis of the receptor.
3. The mRNA and protein expression of β-arrestin-2, β-ARK1 and β-ARK2 remained unaltered during the exposure time. However, mRNA and protein level of β-arrestin-1 exhibited a gradual but significant increase during 2-14 h.


5. Over expression of β-arrestin-1 cDNA in hypothyroid astrocytes caused morphological differentiation even in the absence of TH in the medium.

6. Application of endocytic inhibitors could block the endocytosis of β2-AR as well as sustained endosomal ERK activation.

Thus, results indicate that although there is requirement of both β-arrestin-1 and β-arrestin-2 isoforms for the endocytosis of β2-AR, TH selectively up-regulate β-arrestin-1 in astrocytes to facilitate endocytosis of β2-AR, required for endosomal ERK activation to drive the differentiation process.

**Part 2: Exploring the role of these key regulators in DHA-induced astrocytes differentiation**

1. The mRNA and protein expression of β2-AR was increased during DHA-induced astrocytes differentiation.

2. Application of the β2-AR antagonist, ICI-118,551 could block the differentiation process induced by DHA.

3. PKA activity showed an induction after 6 h of DHA exposure to hypothyroid astrocytes, which came down to basal level immediately and continues till 48 h. This induction was sufficient for driving the differentiation process to completion.

4. PKA inhibitor, H-89 could also block the DHA-induced differentiation.

5. pERK and pCREB protein level showed an sustained activation from 6 h onwards upon exposure to DHA.

6. Endocytic inhibitors could block the differentiation of the astrogial cells although the protein expression of all endocytosis regulators like β-arrestin-1, β-arrestin-2, β-ARK1 remained unaltered during the exposure time.
Comparing these two mechanisms, it is suggestive that astrocyte differentiation by both TH and DHA follows the common mechanism of activation of PKA, endocytosis of β2-AR and sustained ERK activation. They differ on their action on the upstream effectors triggering these events. Whereas, TH causes the induction of β-arrestin-1 to facilitate internalisation of the β2-AR, DHA selectively increases the expression of the β2-AR isotype causing higher cytosolic levels of these receptors.