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Pilot Study

ErbB2 and EphA3 as a novel prognostic and therapeutic target of Recurrent Non-functioning Pituitary Adenomas: A pilot study

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Abstract

Non-functioning pituitary adenomas (NFPA) are 30% of all pituitary adenomas. Although benign in nature but they may be invasive and recurrent. Markers of recurrence are needed to guide patient management. Recteptor Tyrosine Kinases (RTK) may sereve as therapeutic marker for recurrence as they can targeted by already available tyrosine kinase inhibitors. To examine differential RTK phosphorylation pattern of recurrent NFPAs, we recruited 20 patients and grouped them as non-recurrent (n=10), and recurrent (n=10). Recurrence was determined by follow-up of 15 years. We then performed a membrane-based antibody array (ARY001B) for the determination of the relative phosphorylation of 49 tyrosine kinases in recurrent NFPAs. As the experiment was replicated on two set of membranes, each tyrosine kinase was represented in quadruples. Student's t-test was performed to compare the means between two groups.

We found ErbB2, PDGFR beta, SCFR, Trk, VEGFR1, VEGFR2, EphA3, and Alk significantly hyperphosphorylated in recurrent NFPAs. Out of these eight hyperphosphorylated tyrosine kinases ErbB2 and EphA3 were 1.6 (p=0.01) and 1.9 (p=0.002) times hyperphosphorylated in recurrent NFPAs. This result indicates that EphA3 may be an effective therapeutic target in recurrent NFPAs.

KeyWords: Non-functioning pituitary adenomas; Recteptor Tyrosine Kinases; phosphorylation

Introduction

Pituitary adenomas are among the most common intracranial tumors with prevalence ranging from 78 to 94 cases per 100000 [1], 68% of which are macroadenoma. These are benign neoplasms which often grow invasively but very rarely progress to true carcinomas [2]. Although classified as benign, they may cause significant morbidity in affected patients due to aberrant hormone secretion, as well as compressive effects on nearby tissues, such as optic chiasm, or the healthy pituitary leading to hypopituitarism. A sizeable proportion of pituitary adenomas (30%) are NFPA. NFPAs are tumors without a clinical evidence of hormone production. Due to their lack of clinically detectable hormonal activity, they tend to present with mass effects. The molecular pathomechanics of NFPA is not well understood. Treatment of these non-functioning tumors is either surgery or radiotherapy but they have high recurrence rate and associated with severe morbidity [3].The determinants of tumor recurrence after surgical treatment for NFPA are largely unknown. We, therefore, planned to evaluate the molecular predictors and determinants of tumor recurrence after surgery.

Reversible phosphorylation of RTKs regulates a variety of biological processes including communication, cell growth, proliferation, differentiation, and apoptosis. Dysregulation of kinase signaling pathways is commonly associated with various cancers [,4,5]. This aberrant regulation may result from hyperactivation of kinases, mutations or defect in the negative feedback. Characterization of phosphorylation status is critical to the elucidation of signal pathways and to the understanding of tumorigenesis.

Therefore identifying abberantly phosphorylated RTKs will provide an important clue to understanding the mechanism of NFPA pathogenesis, they can be used as predictive measures for aggressive behaviour as well as activated kinases may be specifically targeted using small molecule inhibitors.

Methods

Patients: Tumor samples from 20 (with mean age 43±12.6years) male patients of non-functioning pituitary adenoma from the Department of Neurosurgery, PGIMER, Chandigarh, were selected after obtaining an informed written consent and approval by Institute Ethics Committee (NK/1790/PhD/6957). Patients were divided into two groups non-recurrent (n=10) and recurrent (n=10).

Protein Extraction: we extracted protein using 2%SDS, lysis buffer with phosphatase and protease inhibitor cocktail [6] (Roche, Cat.No.11836153001). The concentration of proteins was measured by Bicinchoninic acid assay (Pierce, Waltham, MA Cat #23225). Phosphoproteins were purified using phosphoprotein purification kit (Qiagen, 37101). Isolated phosphoproteins were separted on 10% SDS-PAGE, followed by immunoblotting with anti-phosphotyrosine antibody. The relative phosphorylation levels of 49 RTKs were analyzed (Fig.1**)** using the Human Phospho-RTK Array kit (ARY001B, R&D Systems, Minneapolis, MN, USA), according to the manufacturer's protocol.

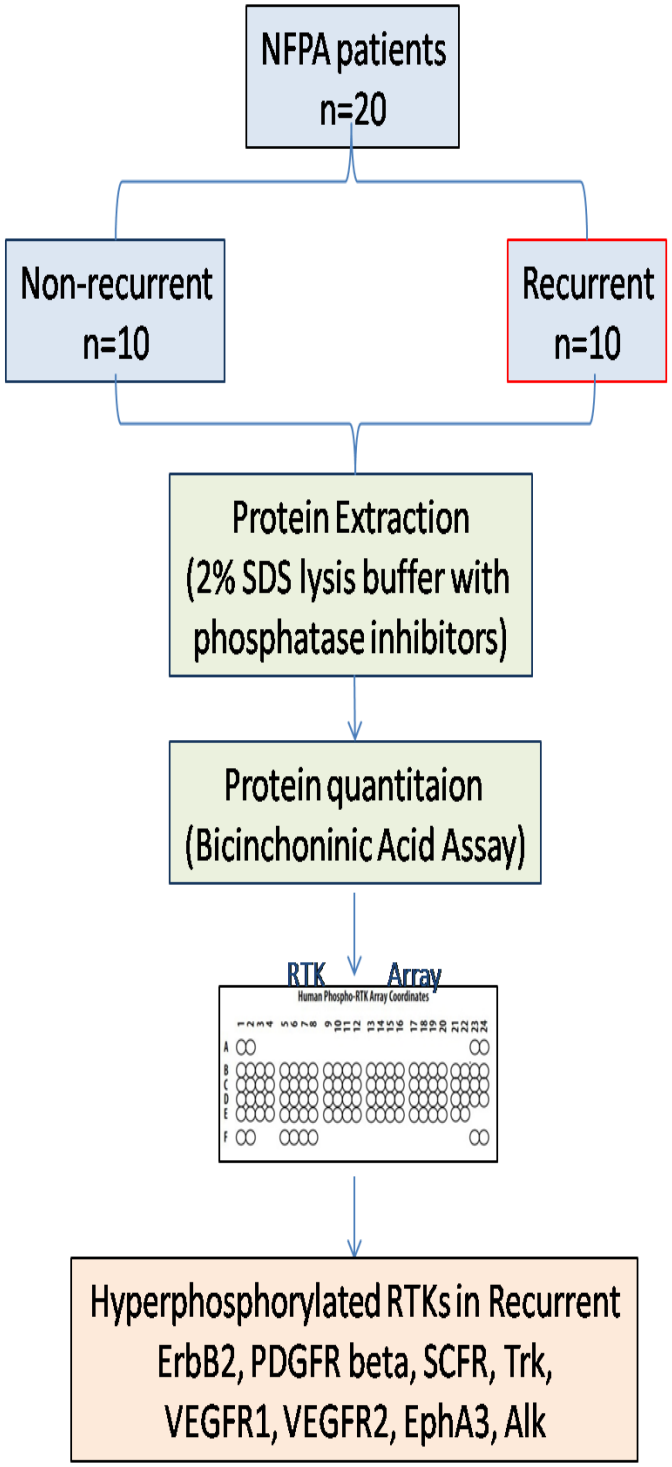

Figure1: Overview of the experimental design and outcome. Flow chart of both RTK array analysis and technical validation.

The levels of phosphorylation were quantified using ImageJ software and normalized to control spots and the background. Data was recorded as mean pixel density of spots and Student's t-test was performed to compare the RTK phosphorylation level between recurrent and non-recurrent group

Results

The quantitative raw data has been submitted to Figshare (RTK_NFPA:https://figshare.com/articles/RTK_NFPA_xlsx/7528430). ErbB2, PDGFR beta, SCFR, Trk, VEGFR1, VEGFR2, EphA3, and Alk were significantly hyperphosphorylated in recurrent NFPAs. ErbB2 and EphA3 were found to be 1.5 and 1.9 times hyperphosphorylated in recurrent adenomas (Fig 2).

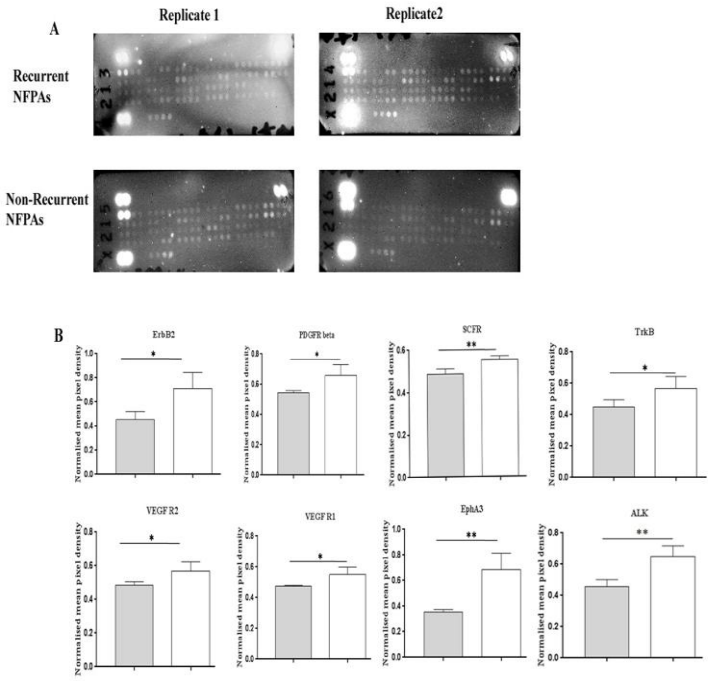

Figure2: Hyperphosphorylation of receptor tyrosine kinases in recurrent NFPAs. RTK array of both replicates 1 and 2 (A) were quantified and ErbB2, PDGFR beta, SCFR, Trk, VEGFR1, VEGFR2, EphA3, and Alk were found to be significantly hyperphosphorylated in recurrent adenomas (B). $p<0.01$(*), $p<0.001$(**).

These results suggest that ErbB2 and EphA3 targeting drugs will be useful agent for recurrent NFPAs.

Discussion

Our study suggest that hyperphosphorylation of EphA3 and ErbB2 may play crucial role in tumor development and recurrence. ErbB2 are overexpressed and regulate tumor behaviour of corticotroph and lactotroph [7]. Additional validational study of these targets in preclinical models will be required to advance their use for prognostication and theraputics Utilization of RTK inhibition could be a turning point for management of recurrent NFPAs.

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Competing interests

There is no conflict of interest

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