Novel NRG1 gene fusions and its tumor associations identified by next-generation sequencing

N. Ptakova MSc 1,2, V. Hajkova MSc1, T. Vanecek PhD1, P. Martinek PhD1. Steiner PhD1

Introduction: The NRG1 gene is localized on the chromosome arms 8p. It codes protein neuregulin 1, a critical signaling protein that mediates the growth and development of multiple organ systems. The NRGs present high affinity to erythroblastic leukemia viral oncogene receptor 1–4 (ERBB 1–4), which belong to the family of transmembrane receptor tyrosine kinases (RTK) (Fig.2) Its aberrant activation, mainly via mutation and fusions, is associated with a wide range of malignancies. It is also believed to be a promising therapeutic target in the near future, similar to NTRK genes. The Archer technology permits the simultaneous detection of both known recurrent fusions as well as previously unidentified fusions at key breakpoints in target genes. Targeted next-generation sequencing is currently used for diagnostics, prognostics and predictive molecular analysis in routine molecular pathology.

Aims: Our study set out to retrospectively examine the NRG1 fusions identified at our institution over a 2-year period, using various NGS panels, detection of novel fusion partners and their association with specific tumor entities.

Materials and Methods: In the years 2017–2018, 2480 different neoplastic tissues were analyzed for the presence of fusion transcripts by targeted RNA sequencing. Fusion Plex Solid Tumor and Comprehensive Thyroid and Lung Kit (ArcherDX Inc., Boulder, CO) was used to construct cDNA library for detection fusion transcripts in 52 and 36 genes, respectively. All positive results were merged with clinical follow – up. We used data mining to seek and classify the NRG1 gene rearrangements in our internal database.

Results: Six NRG1 fusion-positive cases investigated by Fusion Solid Tumor (Archer Dx) panel were identified during 2017 and 2019 at our institution (Table 1). Fusions were identified by RNA-based AMP and confirmed by break-apart fluorescence in-situ hybridization (FISH)and/or primer specific RT-PCR. Five of the six identified fusion genes were unique. Two novel fusions were identified, namely TNC – NRG1 in a kidney papillary carcinoma, and a UNC5D-NRG1 in a prostate acinic carcinoma. The rest four NRG1 gene alteration, which included SDC4-NRG1, CD74– NRG1 and VAMP2-NRG1 fusions, were seen in lung adenocarcinomas (bronchus adenocarcinomas and lung adenocarcinoma). The EGF–like domain which is nessesery to functional gene fusions was always retained (Fig 1).

Conclusions: NRG1 rearrangements occurred predominantly in lung cancers however can be detected across a variety of other cancers. Using a large targeted RNA sequencing panels proved to be efficient in detecting new clinically actionable target.

Figure 1- NRG1 fusion transcripts

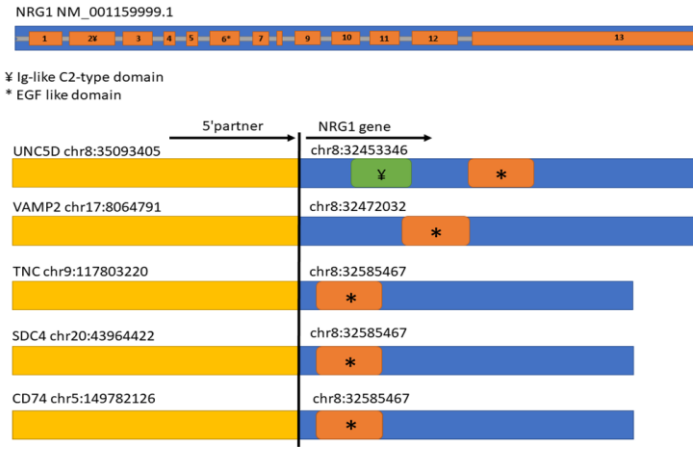

Figure 1 The blue strip is a graphic representation of the NRG1 gene with all 13 exons including exon 2 containing Ig-like C2 type domain (indicated by yen sign) and exon 6 containing EGF-like domain (indicated by asterix). Yeallow-blue strips represent identified in-frame fusion genes consisting of the associated chromosomal partners (yellow), and corresponding part of NRG1 gene with the EGF-like domain and Ig-like C2 domain indicated. (NRG1 fusion partners are not drawn to scale.) All Identified fusion partners had already been described (CD74, SDC4, TNC, VAMP2, UNC5) with SDC4 and CD74 being identified in two of our cases.

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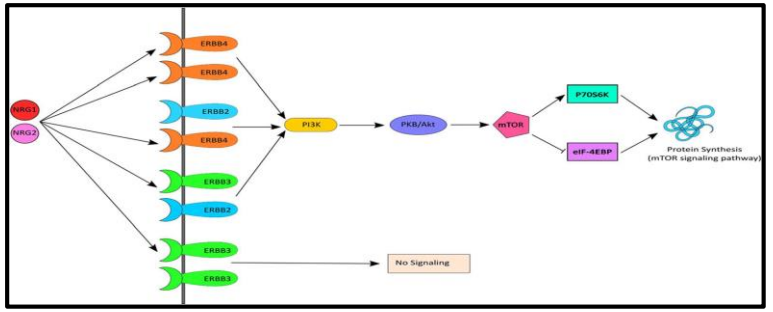

Figure 2 Neuregulin-1 and ErbB4, together, initiate signaling via the PI3K-AKT signaling pathway, which results in activation of mTOR and in turn stimulates protein synthesis

Patient ID	Localization	5' Chrom.	3' Chrom.	5' Gene	3' Gene	5' Breakpoint	3' Breakpoint	Reading Frame	Functional domains present in the NRG1 fusion proteins	Supporting Reads (#)	Supporting Start Sites	Fusion Type	FISH NRG1ba	RT-PCR
1096911/19	Bronchus, Adenocarcinoma	5	8	CD74	NRG1	chr5:149782126	chr8:32585467	in frame	EGF-like	1677	124	InterChromosomal	0	+
1117329/19	Bronchus, Adenocarcinoma	20	8	SDC4	NRG1	chr20:43964422	chr8:32585467	in frame	EGF-like	18	12	IntraChromosomal	0	+
6500360/19	Kidney, Papilary carcinoma	9	8	TNC	NRG1	chr9:117803220	chr8:32585467	in frame	EGF-like	391	66	InterChromosomal	0	+
1109929/18	Bronchus, Adenocarcinoma	17	8	VAMP2	NRG1	chr17:8064791	chr8:32472032	in frame	EGF-like	421	126	IntraChromosomal	0	+
6501288/18	Prostate, Acinar adenocarcinoma	8	8	UNC5D	NRG1	chr8:35093405	chr8:32453346	in frame	Ig-like C2-type, EGF-like	22	10	InterChromosomal	0	+
1121291/19	Lung, Mucinous carcinoma	20	8	SDC4	NRG1	chr20:43964422	chr8:32585467	in frame	EGF-like	369	92	IntraChromosomal	0	+

Table 1 Details of the fusion transcripts found in this study. Exact Breakpoint, reading frame, conserved domains, and other analytical details are mentioned. Confirmation results are also included. We found out that the FISH break-apart probe is not working well in many cases, however, specific primer RT -PCR was supportive in all cases.

Affiliation
1Molecular Genetics Department, Biopticka Laborator s.r.o., Pilsen, Czech Republic
2Second Faculty of Medicine, Charles University, Prague, Czech Republic