**Please nominate 1 category that best fits your submitted abstract:**

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**Characterising *in-vitro* established cancer associated fibroblasts in High Grade Serous Ovarian Cancer**

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**Background and Aims:** High Grade Serous Ovarian Cancer (HGSOC) is the most common and aggressive ovarian cancer subtype with a poor prognosis. The tumour microenvironment (TME) is an emerging therapeutic target in HGSOC. Cancer associated fibroblasts (CAFs) – the most abundant cell type within the TME – interact with tumour cells and other TME components to drive tumour growth and metastasis. The diverse origin and activation mechanisms of CAFs gives rise to its heterogenous population with a lack of specific markers. Hence, it is of utmost importance to understand the sub-types and their functions to ultimately improve CAF targeted therapy in HGSOC. The main objective is to establish and characterise *in-vitro* generated CAFs.

**Methods:** immortalised normal ovarian fibroblasts (iNOFs) were used to establish *in-vitro* CAFs through two stimulation methods. Method 1: iNOFs were treated with 3ng/mL TGF-β1 and 30µM LPA at 6-, 12-, 24- and 48hr timepoints. Method 2: Conditioned media (CM) was obtained from 3 different HGSOC cell lines (OVKATE, OVSAHO and CAOV3) after 24hr incubation and concentrated X10. The X10 concentrated CM was then used to stimulate iNOFs at 6-, 12-, 24- and 48hr timepoints. Fibroblasts from each time point and stimulation method were characterised by morphology (IncuCyte) and a CAF-associated protein expression (Western Blot) panel consisting of α-smooth muscle actin (α-SMA), fibroblast activation protein (FAP), fibroblast specific protein-1 (FSP-1), caveolin-1, platelet derived growth factor receptorβ (PDGFRβ), vimentin, integrin β1.

**Results:** Both stimulation methods induced a CAF phenotype at 48hrs post-treatment, with a significant increase in α -SMA, PDGFRβ and significant decrease in caveolin-1. CAF phenotype was further confirmed by elongated fibroblast morphology and contractility.

**Conclusions and Significance/Impact:** This work has successfully established an *in-vitro* CAF phenotype to be used in HGSOC TME research. Future work will engage RNA-sequencing to further investigate the transcriptomic differences in both stimulation methods.

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High Grade Serous Ovarian Cancer (HGSOC) is the most common and aggressive ovarian cancer subtype with poor prognosis. Cancer associated fibroblasts (CAFs) are abundantly present within the tumour microenvironment (TME) and is an emerging therapeutic target as it interacts with tumour cells and other TME components to drive tumour growth and metastasis. The diverse origin and activation mechanisms of CAFs gives rise to its heterogenous population with non-specific markers which remain a challenge. Hence, understanding the sub-types and their functions is essential to improve CAF targeted therapy in HGSOC. The main objective is to establish and characterise *in-vitro* generated CAFs.