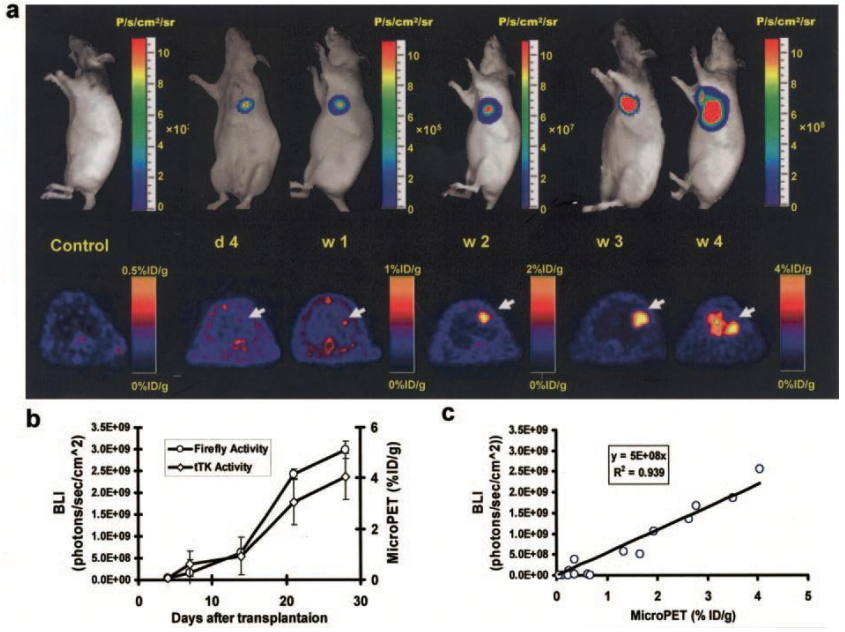
小动物活体光学成像技术在心血管疾病研究中的应用

Revvity小动物活体光学成像技术已在生命科学基础研究、临床前医学研究及药物研发等领域得到广泛应用。在众多应用领域中，心血管疾病是目前研究的热点领域之一。常用于心血管研究的光学标记方法包括：1. 利用荧光虫荧光素酶（Firefly Luciferase）或荧光蛋白作为报告基因，通过标记特定基因而研究心血管疾病相关基因在心血管疾病中的作用；2. 通过外源注射功能性荧光探针，观测心血管疾病发展过程中的分子变化。应用小动物活体光学成像技术进行心血管疾病研究主要集中在三个方面：1. 应用生物发光技术，研究细胞治疗心血管疾病效果；2. 应用功能性荧光探针，了解疾病发展的分子机理和药物治疗心血管疾病的效果；3. 心血管疾病相关基因的作用及其治疗。下面将对一些具体实例进行阐述：

一．应用生物发光技术，研究细胞治疗心血管疾病效果

通过细胞示踪，可以了解细胞在心脏的分布并且了解心脏的调控机制。新型的报告 基因（triple-fusion，TF）包括萤火虫荧光素酶，单体红色荧光蛋白和缩短的胸苷激酶。TF 报告基因体外转染鼠胚胎干细胞，然后注射胚胎干细胞到裸鼠心肌层。Revvity的 IVIS 成像系统显示 的生物发光成像和正电子发射层断成像（PET）有很强的相关性，并且能够监测移植后胚 胎干细胞存活，增殖和迁移 （下图）。

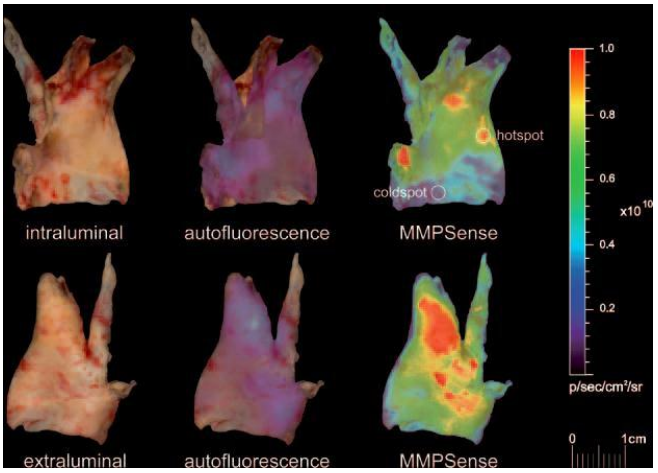


（Lee & Wu, Stem Cell Migration, 2011）

上图： 移植含有 TF 报告基因的胚胎干细胞到无胸腺的裸大鼠心脏。使用生物发光（a，上图）和 PET（a，下图）纵向监测 胚胎干细胞在心脏内移植后的存活和增殖。（b & c），定量分析生物发光和 PET 成像的强度，之间有很强的关联性。

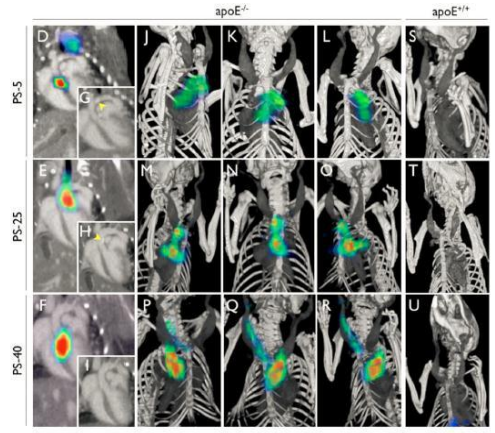
二．应用功能性荧光探针，了解疾病发展的分子机理和药物治疗心血管疾病效果

血管疾病发展过程中伴随着分子表达的改变，比如基质金属蛋白酶的表达特异性升高，因此通过观测体内分子的改变能够判断心血管疾病的发展和治疗效果。研究发现基质金属蛋白酶 (Metalloproteinases, MMPs) 在动脉粥样硬化中高度聚集，应用 Revvity的 MMPSense 探针并通过 IVIS Spectrum 仪器成像显示人动脉粥样硬化中金属蛋白酶的活性。下图说明，应用 MMPSense 探针能够检测人颈动脉内膜切除手术的动脉粥样硬化斑块。

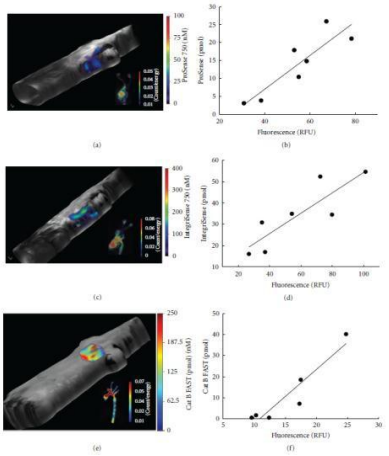


(Wallis et al, Circulation. 2009)

在 apoE−/− 心血管疾病小鼠模型中，大动脉根的组织蛋白酶活性（Protease Sensor，PS）升高 （左下图）。使用 Revvity的 Prosense，IntergrinSense 和 CatBFast 探 针分别对心血管疾病中大动脉根的组织蛋白酶和整联蛋白 αvβ3 活性进行观测。如右下图 所示，活体和体外组织的成像结果相一致（使用 Revvity的 FMT 小动物活体荧光断层 成像系统。

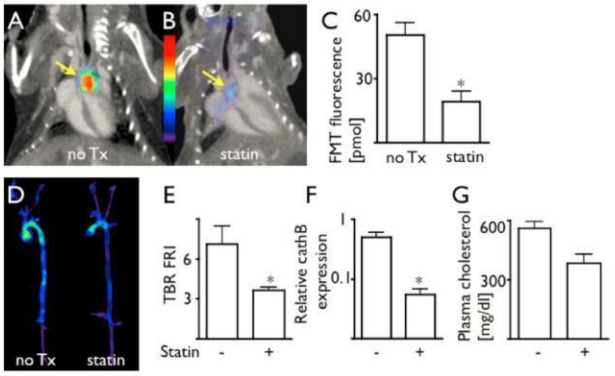


(Nahrendorf et al., Arterioscler. Thromb. Vasc. Biol., 2009）



(Li et al., International Journal of Molecular Imaging, 2012)

Atorvastatin（阿托伐他汀）可以降低炎症性单核细胞的募集和血管细胞粘附分子的表达，而且斑块的组织蛋白酶主要来源自于炎症性单核细胞和血管细胞。如下图所示，Revvity的 FMT 小动物活体荧光断层成像系统显示出 Atorvastatin 能有效治疗 apoE−/− 心血管疾病小鼠模型。通过 Atorvastatin 治疗后，主根动脉的组织蛋白酶活性下降。



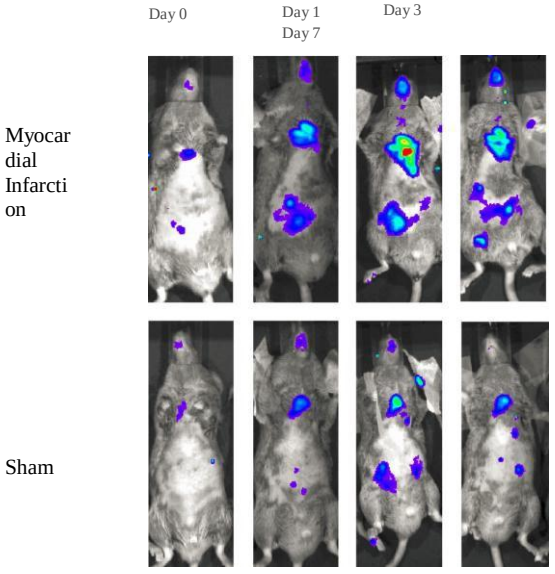
（Nahrendorf et al., Arterioscler. Thromb. Vasc. Biol., 2009）

上图：Atorvastatin 降低 apoE− /−心血管疾病小鼠模型的组织蛋白酶活性，基因表达和胆固醇水平。

三．心血管疾病相关基因的作用及其治疗

利用小动物活体光学成像，能够研究在活体动物水平上心血管疾病相关基因在其发 展过程中的作用。利用生物发光技术进行心血管疾病相关基因的研究，主要用基因-荧光素 酶的共表达载体，通过荧光素酶产生的生物发光信号反映该基因的表达情况，研究该基因 的相关作用。

NF-κB 是细胞核内的转录因子，能被炎症性的细胞因子(TNF, IL-1β, IL-6, IL-8)激活。下图所示，科研人员使用荧光素酶 NF-κB 转基因小鼠研究心肌梗塞，冠状动脉结扎小鼠 (Myocardial infarction) 中的 NF-κB 基因表达水平相对于假性手术处理的对照小鼠(Sham)显著升高。研究表明 NF-κB 基因在心肌梗塞的发展中起了重要的作用。

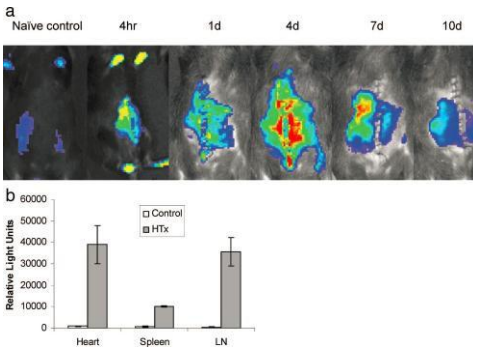


NF-κB 转基因小鼠的生物发光信号代表 NF-κB 基因表达情况。心肌梗塞诱导 NF-κB 基因水平升高，Sham 代表假手术处理的对照小鼠。Revvity的IVIS 成像系统成像。

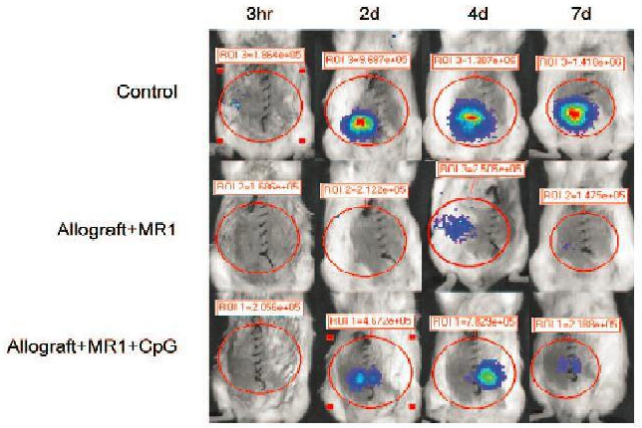
（Tillmans et al, Biophys. Biochem. Res. Comm., 2006）

使用 NF-κB 转基因小鼠进行同种异体血管化心脏移植的研究。Revvity的 IVIS 成像系统显示野生型 Balb/c 心脏同种异种移植到萤火虫荧光素酶标记 NF-κB 转基因 C57BL/6 小鼠，NF-κB 的基因表达水平 明显提高，说明 NF-κB 在同种异体移植中被激活(下图 A)。CD154 抗体（MR1）治疗可以 有效地提高同种异体血管化心脏移植后受体小鼠的耐受性和存活率，并且抑制 NF-κB 基因 表达水平。然而服用 CpG DNA 结合 Toll 样受体 9，提高 NF-κB 表达水平并且抑制 CD154 抗体治疗效果 （下图 B）。

(A)

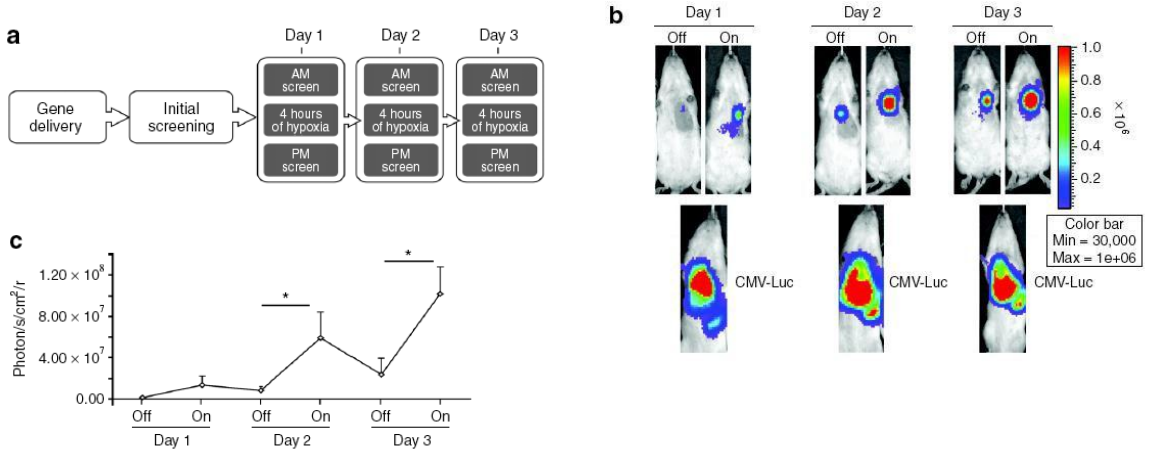


(B)



(Ma et al, Transplantation, 2008)

荧光素酶标记的低氧报告基因载体系统（Double oxygen-sensing vector system, DOSVS）心肌内注射到小鼠心脏。通过间断性缺氧实验，研究人员使用 Revvity的IVIS 成像系统观 测低氧报告基因表达的变换。下图所示在心脏缺氧（On）情况下，低氧报告基因表达升 高；然而在常氧条件（Off）下，低氧报告基因表达下降。实验证明低氧报告基因可以用来 监测心脏缺氧或者局部缺血的产生和发展。



Fomicheva *et al*, Molecular Therapy, 2008

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